

THE EFFECT OF EXERCISE ORDER ON TESTOSTERONE AND  
CORTISOL RESPONSES TO LOWER AND UPPER BODY  
RESISTANCE TRAINING EXERCISES

by

Jason D. Miller

A dissertation submitted to the faculty of  
The University of Utah  
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Exercise and Sport Science

The University of Utah

December 2010

Copyright © Jason D. Miller 2010

All Rights Reserved

# The University of Utah Graduate School

## STATEMENT OF DISSERTATION APPROVAL

The dissertation of Jason D. Miller  
has been approved by the following supervisory committee members:

<u>Patricia Eisenman</u>	, Chair	<u>8/2/2010</u> Date Approved
--------------------------	---------	----------------------------------

<u>Bradley Hayes</u>	, Member	<u>8/2/2010</u> Date Approved
----------------------	----------	----------------------------------

<u>Robert Kraemer</u>	, Member	<u>8/2/2010</u> Date Approved
-----------------------	----------	----------------------------------

<u>William Kraemer</u>	, Member	<u>8/2/2010</u> Date Approved
------------------------	----------	----------------------------------

<u>Janet Shaw</u>	, Member	<u>8/2/2010</u> Date Approved
-------------------	----------	----------------------------------

<u>Daniel Williams</u>	, Member	<u>8/2/2010</u>
------------------------	----------	-----------------

and by Barry Shultz, Chair of  
the Department of Exercise and Sport Science

and by Charles A. Wight, Dean of The Graduate School.

## ABSTRACT

The purpose of this study was to determine the effect of resistance exercise order on circulating testosterone (T) and cortisol (C). A secondary purpose was to assess the effect of exercise order on volume (sets x repetitions) and the perception of the volume as measured by session rating of perceived exertion (SRPE).

Adult males with at least 1 year of resistance training and minimum strength to body mass ratio of 1:1 for the bench press (BP) and 2:1 for the leg press (LP) were recruited for the study. During session 1, participants were familiarized with the BP and LP and tested for the maximum load that could be lifted for the two exercises. On two separate sessions separated by 72 hours, participants performed both the BP-LP (session 2) and the LP-BP (session 3) order. For both exercises a load 73.5% of one repetition maximum was lifted to failure over 4 sets. Exercises were separated by 5 minutes and sets by 2 minutes. Blood samples were taken and analyzed at pre-, mid- and postsession. T and C values were assessed as plasma volume (PV) corrected and uncorrected.

There was not a significant difference for the order by time interaction for PV corrected or uncorrected T and C. Total volume was not significantly different ( $p=0.61$ ) between the UB-LB ( $62\pm 7$  reps) and LB-UB ( $61\pm 7$  reps) orders. SRPE was not significantly different between the two orders ( $p=0.22$ ). The order by time interaction for lactate was not significant ( $p = 0.14$ ).

There does not appear to be an affect of resistance the exercise order of LP and BP on T and C. The exercise orders resulted in the same exercise volume and lactate responses which in turn resulted in no interaction in T and C between the UB-LB and LB-UB exercise orders. The difference in working skeletal muscle between the BP and LP may not have been great enough to produce a significant interaction for T and or C. More research is needed to determine if exercise order may be important using other popular resistance exercises.

## TABLE OF CONTENTS

ABSTRACT.....	iii
LIST OF TABLES .....	viii
LIST OF FIGURES .....	ix
ACKNOWLEDGMENTS .....	xi
Chapters	
1 INTRODUCTION .....	1
The Effect of Testosterone on Skeletal Muscle Hypertrophy .....	2
The Effect of Cortisol on Skeletal Muscle Hypertrophy .....	3
Importance of Plasma Volume on Measuring Testosterone and Cortisol .....	4
Acute Program Variables for Optimally Increasing Testosterone .....	5
The Effect of Exercise Order on Testosterone and Cortisol .....	6
Research Questions and Hypotheses .....	8
Significance of the Study .....	9
Delimitations .....	10
Limitations .....	11
2 REVIEW OF LITERATURE .....	12
Introduction.....	12
Testosterone: Background .....	12
Exercise-Induced Elevations in Testosterone .....	14
Total Versus Free Testosterone .....	15
Direct Effects of Testosterone on Skeletal Muscle.....	16
Indirect Effects of Testosterone on Skeletal Muscle .....	19
Resistance Exercise and its Influence on the Androgen Receptor in the Presence of Testosterone.....	19
Cortisol: Background.....	21
Total Versus Free Cortisol .....	23
The Effect of Cortisol on Skeletal Muscle.....	23
Testosterone and Cortisol Kinetics in Response to Training.....	24
Nonexercise Factors Affecting Testosterone and Cortisol Concentrations .....	26

	Diurnal Fluctuations.....	26
	Body Fat.....	27
	Diet.....	28
	Age.....	29
	Training Status .....	30
	Hydration .....	32
	Changes in Plasma Volume .....	33
	The Effect of Resistance Training Program Variables on Testosterone and Cortisol Concentrations .....	33
	Intensity.....	34
	Volume, Rest Period Length and Repetitions to Failure .....	35
	Resistance Training Modality .....	37
	Change in Testosterone and Cortisol Concentrations During and After a Bout of Resistance Exercise.....	38
	Testosterone and Cortisol Responses to Body Segment Specific Resistance Exercises .....	40
	Resistance Exercise, Lactate Concentrations, and Session Rating of Perceived Exertion.....	43
	Summary .....	45
3	PROCEDURES AND METHODS.....	47
	Participants.....	47
	Study Design.....	48
	Procedures.....	48
	Session 1: Familiarization and Maximum Strength Testing.....	50
	Sessions 2 and 3: UB-LB and LB-UB Exercise Orders .....	53
	Resistance Exercises .....	54
	Volume.....	55
	Blood Sampling .....	56
	Assessments and Instruments .....	56
	Session Rating of Perceived Exertion.....	57
	Statistical Analysis and Sample Size Estimation.....	58
4	RESULTS AND DISCUSSION .....	59
	Results.....	59
	Discussion .....	67
5	SUMMARY, CONCLUSION, AND FUTURE RESEARCH RECOMMENDATIONS .....	81
	Summary .....	81
	Conclusion .....	82
	Limitations .....	82

Future Research Recommendations.....	86
Appendices	
A 3-DAY FOOD RECALL .....	88
B IRB CONSENT FORM .....	90
C SESSION RATING OF PERCEIVED EXERTION .....	94
REFERENCES .....	95



## LIST OF TABLES

Table	Page
3.1    Sets and Repetitions for Session 1, Familiarization and Maximum Strength Protocol .....	51
4.1    Descriptive Data for the Sample of 9 Adult Men .....	60
4.2    Bodyweight, Hours Slept, Session Start Time and Dietary Values Over the Course of the Study .....	62
4.3    Hemoglobin (Hb), Hematocrit (Crit) and Changes in Plasma Volume (PV%Δ) for Both the UB-LB and LB-UB Exercise Orders .....	64
4.4    The Total Exercise Volumes for the UB-LB and LB-UB Orders Were not Significantly Different .....	66

## LIST OF FIGURES

Figure	Page
3.1. Timeline for the study.....	50
4.1 Testosterone concentrations at pre, mid and post both the UB-LB and LB-UB exercise orders. ....	63
4.2. Cortisol concentrations pre, mid and post both the UB-LB and LB-UB exercise orders. ....	63
4.3. Plasma volume corrected testosterone concentrations pre, mid and post UB-LB and LB-UB exercise orders.....	65
4.4. Plasma volume corrected cortisol concentrations pre, mid and post both UB-LB and LB-UB exercise orders.....	66
4.5. Session Rating of Perceived Exertion for the UB-LB order and the LB-UB order. A score of 10 is classified as “maximal” and 1 is classified as “very, very easy.” Values were not significantly different between sessions 2 and 3.....	68
4.6. Lactate concentrations pre-, mid- and postsession for both the UB-LB exercise order and the LB-UB exercise order.....	68
4.7. Intraindividual difference in T pre- to midsession and pre- to postsession for both the UB-LB exercise order and the LB-UB exercise order. A 95% Confidence Interval is represented by the bars around each mean. ....	70
4.8. Individual difference in T between the UB-LB exercise order and the LB-UB exercise order (3-2). Individual change in T is post-pre session plasma volume uncorrected T. A positive value indicates the UB-LB order produced a greater T response. The reported T values had the 5.1% average interassay error removed for each value. ....	70
4.9. Correlation between the individual differences in plasma volume uncorrected T between sessions 2 and 3 and the individual difference in total volume from sessions 2 and 3.....	72

4.10.	T concentrations for each individual at every time point. The dotted line represents the group mean. ....	72
-------	---	----

## ACKNOWLEDGMENTS

I would like to thank my family for their unwavering support over the 14 years of school. My father for his patience and support in letting me find my way and my mom for her encouragement. I would also like to thank the friends, colleagues and mentors who made my journey everything I thought it would be. Certainly every faculty member at the University of Utah: Dr. Barry Shultz, Dr. Dan Williams, Kerry Jacques, Dr. Bradley Hayes, Dr. Jim Martin, Dr. Dave Symons, Dr. Russ Richardson, Shannon Mulder and Dr. James Hannon and faculty at other institutions, Dr. William Kraemer and Dr. Robert Kraemer, have all been an important part of my professional journey, I thank you for all your time, patience and support. I would like to especially thank Dr. Janet Shaw for taking me in when I had nowhere to go professionally and understanding my passions and interests and letting me pursue them. Your influence in my doctoral education was invaluable.

I also have made wonderful friendships that will last a lifetime, people I have been blessed to know. Some have impacted me professionally, some personally and some both but all have been an important part of my experience and education at the U of U, Mike, Josh, Abby, Chad, Badar, Josh, Kary, Arwen, Nick, Christine, Shauna, Ted, Melissa, Steve I., Steve E., Bernie, Andrea, Cheryl, Jon and Tim. I want to especially thank Traci Thompson for her support and unwavering friendship over the course of the

last 4 years. Besides Dr. Eisenman no one person has been more important in my professional journey than Traci.

I could not have done this project or made it through this program without Dr. Eisenman. I cannot say enough how much I have appreciated your mentorship, patience with me as I trudged through my dissertation, job searching and personal challenges. I could never thank you enough.

I want to thank my beautiful wife, Kristin. I love and appreciate you and thank you for your unwavering support to let me pursue my dreams and interests at the expense of your own interests and at times your health. My prayer is that now will be your time to explore your interests, dreams and ambitions.

Lastly and most important, I want to thank the Lord for His guidance and opportunities to pursue what he put in my heart to do. I feel truly blessed that I get up each morning and do what I love. I pray that my career in academia would be used to help and be a blessing to others.

.

## CHAPTER 1

### INTRODUCTION

The increase in muscle contractile protein resulting in muscle hypertrophy is the goal of many resistance exercise programs. Resistance exercise programs that optimally manipulate the training variables including volume, intensity, and rest period duration consistently result in muscle hypertrophy (Ahtiainen et al., 2003; Beaven et al., 2008; Buresh et al., 2009; Kraemer et al., 1990; Kraemer & Ratamess, 2005). However, one training variable, exercise order, has received less attention. While researchers have not extensively examined the importance of resistance exercise order, coaches and the lay press (David, 2007; Fitzgerald, 2003; Johnson, 2010) frequently suggest that performing resistance exercise using the larger muscles in the hips and thighs first, followed by exercises using the smaller muscles of the shoulders and arms facilitates better hypertrophy of the smaller muscle groups. The increase in hypertrophy in the smaller muscle mass is attributed to exercising in a better systemic anabolic milieu created from large muscle mass exercise (Hansen et al., 2001; Kraemer et al., 1990). However, there is little evidence supporting the notion that the ordering of resistance exercise influences the anabolic milieu and skeletal muscle hypertrophy.

The logic of performing large muscle mass resistance exercise first in a resistance exercise bout (REB) is based upon the belief that large muscle exercise will create an anabolic milieu that will foster small muscle hypertrophy. Evaluation of this belief about

the creation of an optimal anabolic milieu for small muscle hypertrophy is contingent upon being able to quantify the anabolic environment. While there are many hormones that contribute to the anabolic environment, such as growth hormone, insulin like growth factor-1 and insulin, currently there is no better marker of changes in the systemic anabolic milieu than the hormone testosterone (T) (Ahtiainen et al., 2005; Bhasin et al., 1996; Häkkinen et al., 1988; Hansen et al., 2001; Sinha-Hikim et al., 2002). Since the magnitude of the anabolic milieu depends upon the concentrations of catabolic hormones as well as anabolic hormones, when assessing the potential effects of a REB on the anabolic milieu, consideration must also be given to hormones that promote a catabolic activity. Catabolic activity could potentially negate the anabolic influence of T, through competition for androgen receptor binding sites and the proteolysis of skeletal muscle. Among the catabolic hormones, the primary hormone is cortisol (C). Therefore, to examine the anabolic milieu created by large muscle resistance exercise, both T and C levels should be assessed.

#### The Effect of Testosterone on Skeletal Muscle Hypertrophy

Testosterone (T), found both in men and women and throughout the lifespan, is responsible for the regulation, promotion, and maintenance of many of the body's tissues including skeletal muscle. Therefore, it is no surprise that T is one of the most studied hormones when it comes to skeletal muscle hypertrophy (Beaven et al., 2008; Bhasin et al., 1996; Hansen et al., 2001; Izquierdo et al., 2001) and maximum strength (Bhasin et al., 1996; Fry et al., 2000; Häkkinen et al., 1987; Izquierdo et al., 2001; Kraemer et al., 1991). The positive effects of T on muscle size and strength have been shown by many researchers including a classic study by Bhasin and colleagues, (1996) wherein

supraphysiological doses of injected T in untrained males significantly increased muscle cross sectional area of the triceps ( $3579 \pm 260 \text{ mm}^2$  to  $4003 \pm 229 \text{ mm}^2$ ) and quadriceps ( $9067 \pm 398 \text{ mm}^2$  to  $9674 \pm 472 \text{ mm}^2$ ) even without the addition of exercise. Häkkinen et al. (1987) reported that changes in baseline biologically free testosterone (FT) levels over 2 weeks were moderately correlated with changes in power during both high volume ( $r = 0.63$ ,  $p < 0.05$ ) and low volume strength training ( $r = 0.58$ ,  $p < 0.05$ ). Furthermore, the strength of the relationship is strong ( $r = .84$ ,  $p < .01$ ) between changes in isometric force and basal T concentrations in strength trained males (Ahtiainen et al., 2003). Despite some research to the contrary (Guezennec et al., 1986; West, Burd, Staples et al., 2010), the overwhelming evidence suggests that T is a primary hormone in muscle hypertrophy and strength development (Ahtiainen et al., 2003; Beaven et al., 2008; Bhasin et al., 1996; Häkkinen et al., 1988; Sinha-Hikim et al., 2002). A more detailed description of the mechanisms of T concentrations on the promotion of skeletal muscle hypertrophy will be explored in Chapter 2.

### The Effect of Cortisol on Skeletal Muscle Hypertrophy

Instead of an increase in T, muscle hypertrophy and increased strength could also result from a drop in total cortisol (C). Cortisol is the catabolic hormone responsible for muscle structural protein degradation to amino acids which are used for gluconeogenesis. Either maintenance or increases in circulating T concentrations along with a drop in C could be an important factor in promoting muscle hypertrophy following either an acute REB or a chronic resistance training program (Kraemer et al., 2009). The notion that declining C levels may promote muscle hypertrophy is consistent with the observation that when measured after chronic resistance training, resting C concentrations decline



(McCall et al, 1999; Staron et al., 1994). Even while total work increases during chronic resistance training, C concentrations after a REB start to decline after about 6-8 weeks of training (Staron et al., 1994). It is interesting that at the same 6-8 week time point, changes in muscle cross sectional area typically start to appear, supporting the hypothesis that reductions in C may contribute to muscle hypertrophy (Staron et al., 1994). Furthermore, studies using a post-REB meal or supplement high in glucose, have reported reductions in C concentrations post-REB and increased muscle hypertrophy when compared with situations where participants do not receive a postworkout meal or supplement as part of the training routine (Kraemer et al., 1998; Tarpenning et al., 2001). However, the degree to which muscle hypertrophy occurs as a direct result from a reduction in circulating C is still undetermined. Like T, a more detailed exploration of C and its effects on skeletal muscle will be considered in Chapter 2.

#### Importance of Plasma Volume on Measuring Testosterone and Cortisol

There is some debate as to whether acute REB-related changes in circulating T and C result from changes in hormone production, decreased clearance by the liver (in the case of T), or an exercise-induced plasma volume shift resulting in a greater exposure of the intracellular hormone receptors to the circulating hormones (Ježová et al., 1985; Kraemer & Ratamess, 2005). For example, Kraemer and colleagues (1992) and McCall and colleagues (1999) reported significant prepost changes in T after a REB. However, after accounting for the REB-related plasma volume shifts by using measurements of hemoglobin and hematocrit and the equation developed by Dill and Costill (1974), the significant REB-related increases in postexercise T concentrations disappeared. Even if REB-related increases in plasma concentrations of T and C concentrations are not from

increases in hormone production but rather exercise-related reductions in plasma volume, the result of the REB is an increase in exposure of intracellular steroid receptors to circulating hormones which may be important in mediating the effects of the particular hormone on its target tissue (Gotshalk et al., 1997; Kramer, Kilgore et al., 1992). Therefore both plasma uncorrected and corrected values for REB-related hormone concentrations may be physiologically relevant and should be reported.

### Acute Program Variables for Optimally Increasing Testosterone

To effectively isolate the effect of exercise order on T and C concentrations, the selection of the optimal combination of volume, intensity and rest interval to change circulating levels of T and minimize subsequent increases in C must be identified. Fortunately the optimal values for volume, intensity and rest interval have been investigated (Ahtiainen et al., 2003, 2005; Crewther et al., 2008; Kraemer et al., 1990).

A consistent result in the literature, when assessing resistance training programs, is that a program using a high volume (~40 repetitions per exercise), moderate load (~70% of 1-RM) and short rest periods between sets (30s-2min) produces significant increases in T and (Ahtiainen et al., 2005; Crewther et al., 2008; Häkkinen et al. 1993; Jürimäe et al., 1990; Kraemer et al, 1991). A significant increase in T resulting from the high volume, moderate load, and short rest period program is primarily from the greater total work performed compared with a low volume, high intensity program (Crewther et al., 2008; Gotshalk et al., 1997; Schwab et al., 1993; Smilios et al., 2007).

Along with volume, intensity and rest interval, the mode of training may also be important when analyzing the response of T and C to a REB. Changes in T and C have been investigated using different muscle contraction types including isometric (Häkkinen

et al., 1995, 1998), and power exercises (Volek et al., 1997). However, the use of these different modalities has yielded conflicting results or results that may not be applicable to the type of dynamic resistive exercises most commonly performed in the gym (Baechle, 1994; Heyward, 2006) and studied in the research literature (Durand et al., 2003; Häkkinen et al., 1987; Kraemer et al., 1991, 1992). Therefore, this study will focus on dynamic resistance exercises which are commonly used as the resistance training modality in lay and professional settings.

### The Effect of Exercise Order on Testosterone and Cortisol

Increases in T could be a result of a greater reliance on type II fibers either through a training planning strategy of fatigue (high volume) exercises or high intensity (>80% of 1-RM) exercises (Fahey et al., 1976) or both (Schwab et al., 1993). Based upon the size principle of motor unit recruitment (Zatsiorsky & Kraemer, 2006), fatiguing the intermediate (size and speed) motor units with a high total training volume and moderate intensity load will result in the recruitment of larger motor units to accomplish the task (Zatsiorsky & Kraemer, 2006). Furthermore, if intensity is increased by using a greater load, type II fibers will also be recruited in order to accomplish the task because type I fibers alone will not be sufficient. The latter approach, using higher intensity loads, has produced some significant increases in T as well, although the high volume approach appears to more consistently raise T (Kraemer et al., 1990, 1991). Additionally, the amount of muscle mass (Hansen et al., 2001; Kraemer, Fry et al., 1992) recruited during an acute bout or a chronic resistance training program will significantly impact the degree of catabolic (Crewther et al., 2008) as well as anabolic (Crewther et al., 2008; Schwab et al., 1993) hormonal response. Since in humans, a greater portion of the body's total

muscle mass is located in the LB as compared to UB (de Leva, 1996), it is easy to conclude, as is done in the lay literature, that LB strength exercises will produce a greater T response than the smaller UB exercises.

Despite the many resistance training studies in the literature, only a few have quantified the differences in the T and or C response between LB and UB resistance exercises and their ordering. In a study with young men (mean age = 26.5 yrs) performing acute bouts of UB and LB isometric resistance exercises of equal volumes on different days, significant postexercise increases in T were observed in the LB but not the UB bout (Häkkinen et al., 1998). Similar results have been seen in a 9-week resistance training study (Hansen et al., 2001), where the addition of LB with UB resistance training elicited a trend toward a greater T response than UB training alone. However, neither study statistically analyzed exercise order and the T and C response.

Additionally, although the REBs used by Häkkinen et al. (1998) did use a multiple joint large muscle mass exercise, the contraction type was isometric and not dynamic. In the training study by Hansen et al. (2001), only a single joint dynamic exercise was used for the UB construct and a multiple joint dynamic exercise was used for the LB construct. Since neither study directly compared separate LB and UB REBs or the effect of the order of LB and UB resistance exercises during a REB on circulating concentrations of T and C further research that specifically examine exercise order is required. Furthermore, the use of maximal voluntary isometric and single joint dynamic contractions may not be applicable to dynamic REBs used by healthy strength athletes and fitness participants, so again further research is warranted.

If there is an effect of T on volume-load leading to muscle hypertrophy, it may be through an effect on the perception of the training load. If the participant perceives the training load as easy, as a result of high circulating T concentrations, the ability to perform more work may occur. The session rating of perceived exertion has been shown to be a valid and reliable tool for evaluating the participant's perception of a training load. Any change in SRPE between REBs with differing exercise order and differing T concentrations would allow for examination of the T concentration exercise perception relationship. (Dey et al., 2004). Such information would be of interest since there is a dearth of literature on the potential mediating effects of T concentrations on the perception of the training load.

In summary, the purpose of this study is to examine the T and C responses to the order of LB and UB resistance exercises during a REB. Secondly if there is a significantly greater increase in acute T concentrations as a result of a LB-UB REB or UB-LB REB, will that acute increase in T affect the training volume and or SRPE for the REB?

### Research Questions and Hypotheses

Research Question 1: Is there a significant order by time interaction for the *plasma volume uncorrected* T and C responses to a UB-LB and LB-UB resistance training exercise order?

Hypothesis 1: *There will be a significant order by time interaction for plasma volume uncorrected prepost increase in T.*

Hypothesis 2: *There will not be a significant order by time interaction for plasma volume uncorrected prepost increase in C.*

Research Question 2: Is there a significant order by time interaction for the *plasma volume* corrected T and C responses to a UB-LB and LB-UB resistance training exercise order?

Hypothesis 3: *There will not be a significant order by time interaction for plasma volume corrected prepost increase in T.*

Hypothesis 4: *There will not be a significant order by time interaction for plasma volume corrected prepost increase in C.*

Research Question 3: Is there a significant difference in training volume completed when comparing an UB-LB resistance training exercise order to a LB-UB resistance training exercise order?

Hypothesis 5: *The LB-UB REB will result in a significantly greater volume than the UB-LB REB.*

Research Question 3: Is there a significant difference in perception of the exercise load when comparing an UB-LB resistance training exercise order to a LB-UB resistance training exercise order?

Hypothesis 6: *There will be no difference in the perception of the exercise load between the UB-LB and LB-UB REBs.*

### Significance of the Study

By understanding the responses of T and C during and immediately after a UB-LB and LB-UB REB, the support for or rejection of the assumption made by the lay press that a LB exercise should be performed before a UB exercise for the optimization of post-REB T concentrations will be accomplished. Additionally, this study will provide insight as to the relationship between post-REB T concentrations and resistive exercise volume

and perception of training load as measured by SRPE. Either an improvement in exercise volume or a reduction in SRPE will indicate a possible mechanism for the influence of an increase in T on muscle performance.

In contrast, if neither a significant increase in exercise volume or reduction in SRPE or difference in T is observed for the UB-LB or LB-UB orders is observed, there will be support to reject the hypothesis that the ordering of a LB resistance exercise before an UB resistance exercise positively enhances the anabolic environment.

### Delimitations

This study is delimited as follows:

1. Only young males ( $\leq 35$  yrs) will be selected for this study because of the potential extraneous effect of menstruation on endocrine function and the lack of a strength trained female population. Young, as opposed to older men, will be selected based upon the ability to tolerate the protocol while having a reduced risk of injury.
2. Only two common resistance training exercises, the bench press and leg press, were selected to use in the study. The exercises selected are common multiple joint exercises performed for both the UB and LB. The selected exercises should recruit the majority of the musculature for either the UB or LB.

### Limitations

This study is limited as follows:

1. Participants will self-report sleep and exercise habits to ensure consistency throughout the study. Although participants will be questioned, the sleep and exercise data are self reported and therefore biased.
2. Although given instructions on portion sizes, the food recall data will require the participants to accurately record the serving sizes of foods consumed.
3. Only two blood draws will be taken during the REBs and only a post-REB sample will be taken with no measures of T or C during recovery.
4. Generalization to other strength training routines will be limited since only two exercises will be used along with only one combination of training volume, intensity and rest interval.
5. The study will only be an acute study and therefore the importance of exercise order in muscle hypertrophy and strength responses to chronic training will not be elucidated.

Only the systemic testosterone and cortisol concentrations will be assessed and therefore the order by time interaction of the hormones with their receptors will not be analyzed.



## CHAPTER 2

### REVIEW OF LITERATURE

#### Introduction

The purpose of this review of literature is to a) provide background information on testosterone (T) and cortisol (C) relative to the creation of an anabolic environment for skeletal muscle, b) discuss factors affecting T and C concentrations, c) examine the effect of resistance training program variables on T and C, d) examine the effects of body segment specific resistance exercises on T and C and e) briefly describe the effect of a resistance exercise bout (REB) on systemic lactate concentrations and session rating of perceived exertion.

#### Testosterone: Background

Testosterone is an androgen produced from the action of the hypothalamic-pituitary-gonadal axis. Gonadotropin-releasing hormone (GnRH) is released in pulsatile bursts from the hypothalamus, and it travels via the hypophyseal-portal circulation activating the gonadotrope cells of the anterior pituitary to release luteinizing hormone (LH). Luteinizing hormone is also released in a pulsatile manner into the circulation (Spratt et al., 1988) where it binds with the Leydig cells of the testes and the Theca and postovulatory Luteal cells of the ovaries (Evans et al., 1992; Kraemer & Rogol, 2005). In the testes in particular, a clear relationship has been observed between LH release and an

increase in T production from the Leydig cells 20-40 min later (Spratt et al., 1988; Veldhuis et al., 1987). The Leydig cells produce 3-10mg of T per day, whereas the Thecal and Luteal cells of the ovaries only produce around 0.15mg of T per day (Evans et al., 1992; Kraemer & Rogol, 2005). In addition to LH, the anterior pituitary hormone, follicle stimulating hormone (FSH), contributes to the Leydig cell production of T in the testes by increasing the production of inhibin in the Sertoli cells of the testes and in the Granulosa cells of the ovaries (Hsueh et al., 1987). Additionally, both sexes produce T in small amounts (500 $\mu$ g) from the 17 $\beta$ -Hydroxysteroid Dehydrogenase5-catalyzed metabolism of the adrenal androgen androstenedione. Androstenedione is converted to testosterone in peripheral tissues (Kraemer & Rogol, 2005).

Once released, circulating T may be taken up and metabolized by the enzyme, 5 $\alpha$ -reductase, to dihydrotestosterone (DHT). Dihydrotestosterone is a more potent androgen than T and binds to the cytoplasmic androgen receptor for up to three times longer than T (Zhou et al., 1995). However, only about 5% of circulating T is metabolized to DHT and skeletal muscle has low concentrations of 5 $\alpha$ -reductase enzyme (Hoffman et al., 2009).

In addition to DHT, T is also metabolized to estradiol in peripheral tissues, particularly adipose tissue, by the enzyme aromatase. Circulating concentrations of estradiol and T form a negative feedback loop with the hypothalamus and the anterior pituitary gland (Horton, 1978). Thus, when circulating estradiol and T concentrations are elevated, the hypothalamic production and secretion of GnRH and the anterior pituitary production and secretion of LH are attenuated (Hoffman et al., 2009; Horton, 1978). As T concentrations decline in the circulation there is also a reduction in the aromatase-

catalyzed metabolism of T to estradiol, which allows for an elevated release of GnRH from the hypothalamus and LH from the anterior pituitary gland.

In men, circulating T concentrations both at rest and after exercise are very individualized even in a homogenous group. Normal resting values in males are considered to be  $>320\text{ng/dl}$  (Buresh et al., 2009; Fahey et al., 1976; Rosner et al., 2007). Peaks in circulating T occur immediately after exercise (Häkkinen et al., 1995; Kraemer et al., 1991, 1998). Testosterone concentrations then start to return to baseline/pre-exercise levels within 30 min (Ahtiainen, Pakarinen, Alen et al., 2003; Durand et al., 2003; Kraemer et al., 1990, 1991). Beyond 30 mins of recovery, T concentrations fall below normal concentrations for up to 13 hours after a heavy REB (Nindl et al., 2001).

#### Exercise-Induced Elevations in Testosterone

In males, during rest and recovery from exercise, T is regulated by LH (Häkkinen et al., 1987, 1988; Nindl et al., 2001; Spratt et al., 1988; Velduis et al., 1987). However, LH does not appear to control T concentrations during exercise (Cumming et al., 1987). The cause of elevated T production in response to a REB, in men, has resulted in many hypotheses (Cumming et al., 1987; French et al., 2007; Lin et al., 2001).

One hypothesis is the possibility of muscle steroidogenesis, wherein the muscle was thought to synthesize testosterone through the enzyme  $3\beta$ -hydroxysteroid dehydrogenase. The enzyme  $3\beta$ -hydroxysteroid dehydrogenase converts dihydroepiandrosterone to androstenedione. The enzyme  $17\beta$ -hydroxysteroid dehydrogenase then catalyzes androstenedione to testosterone (Vingren et al., 2008). However, the muscle steroidogenesis hypothesis has been rejected. Muscle biopsy studies reveal a nonsignificant difference in  $3\beta$ -hydroxysteroid dehydrogenase and  $17\beta$ -

hydroxysteroid dehydrogenase before and after a high volume bout of squats (Vingren et al., 2008).

If steroidogenesis does not exist in skeletal muscle, the most plausible hypothesis for increases in T resulting from an REB is sympathetic innervation and stimulation of the adrenal glands and the Leydig cells of the testes (French et al., 2007; Ježová & Vigaš, 1981; Ježová et al., 1985). The fact that women do not see a physiologically significant increase in T after resistance training (Kraemer et al., 1991) is one piece of evidence, albeit indirect, that supports the hypothesis that sympathetic innervation of the Leydig cells in the testes is the prominent source of circulating T during and immediately after an REB in men.

An additional contribution to T production may also come from exercise induced increases in glycolysis leading to an increase in circulating lactate. Increases in circulating lactate have been shown to stimulate Leydig cell T production in rats (Lin et al. 2001; Lu et al., 1997).

#### Total Versus Free Testosterone

All androgen hormones, including T and estradiol, are derived from a nonpolar cholesterol molecule that is soluble in the blood. Therefore, upon T's release into the circulation the majority of T requires a protein carrier. Around 98% percent of T is bound to either sex hormone-binding globulin (SHBG) (35-40%) or albumin (55-60%). Only a small fraction is considered the free (FT) portion of circulating total testosterone (T) (Hayes, 2000). According to the free hormone hypothesis, only the free portion of a nonpolar hormone should be able to influence target tissues. Therefore, only FT should be able to cross the cellular membrane of a tissue and bind with the cytosolic androgenic

receptor. However, there is support that the SHBG bound T also may be pulled into the cells through an endocytotic process involving the plasma cell membrane receptor protein magelin (Hammes et al, 2005; Nakhla et al., 1996). With the discovery of magelin, total testosterone (bound and unbound T) is thought to be a better marker of the mediating effect of T than FT. Additionally, the strength of the positive correlation between total T and muscle strength and hypertrophy are similar (Izquierdo et al., 2001), if not stronger, than the positive correlation between FT and skeletal muscle strength and hypertrophy (Sinha-Hikim et al., 2002). Resting total T concentrations have a strong correlations with type IIa ( $r^2 = .39$ ) and type IIx ( $r^2 = .46$ ) fiber cross sectional area (Staron et al., 1994). Therefore, total T is probably a better marker of T's effect on skeletal muscle hypertrophy than FT. For the remainder of this manuscript any reference to T will be referring to total testosterone (T).

#### Direct Effects of Testosterone on Skeletal Muscle

Even in the absence of resistance training, skeletal muscle size and strength significantly improve as a result of significant increases in circulating T (Bhasin et al., 1996). In a dose-response experiment, T was elevated over 20 weeks by weekly injections of differing doses of testosterone enanthate in the presence of a GnRH agonist (Leydig clamp). Over the 20 weeks fat free mass and muscle volume changes were monitored (Bhasin et al., 2001; Sinha-Hikim et al., 2002). The 300mg/wk dose of testosterone enanthate increased T from a baseline of  $653 \pm 50$  ng/dl to  $1,345 \pm 139$  ng/dl whereas the 600mg/wk dose increased T from baseline levels of  $632 \pm 63$  ng/dl to  $2,370 \pm 150$  ng/dl. Increases in fat-free mass, and quadriceps volume were seen with the 125mg/wk, 300mg/wk and 600mg/wk testosterone enanthate dosages. Significant

increases were seen in quadriceps type I fiber volume, leg power and maximum leg strength for the 300mg/wk and 600mg/wk dosages while only the 600mg/wk dosage produced gains in type II fiber volume (Bhasin et al., 2001; Sinha-Hikim et al., 2002). Changes in the percentage of type I and type II fibers were not observed for any of the dosages, indicating T increases muscle cross sectional area through hypertrophy and not hyperplasia (Sinha-Hikim et al., 2000).

In a similar study, Kvorning, Anderson et al., (2006) restricted endogenous T production from the hypothalamic-pituitary-gonadal axis during 8 weeks of resistance training. The restriction in endogenous T resulted in significant increases in body fat. Isometric strength did improve significantly in the clamped group, but was significantly lower than that of the nonclamped group after the 8 weeks of resistance training (Kvorning, Anderson et al., 2006). Similarly, improvements in lower body lean mass were seen in the clamped group, but the change from baseline was significantly less than that of the nonclamped group. The authors attributed the T independent increase in hypertrophy to other exercise-induced intramuscular factors such as the density of the androgen receptor and IGF-1 concentrations.

Even if some researchers question the importance of T's role in exercise-induced hypertrophy (Kvorning, Anderson et al., 2006; West, Burd, Staples et al., 2010), in an elegant study by Beaven et al. (2008) the importance of exercising in the presence an individualized optimal FT concentration was highlighted. Participants in the study by Beaven and colleagues first performed four different loading schemes in order to determine which loading scheme produced the maximal and minimal FT response for each individual. Participants then completed 3 weeks of resistance training, using the

loading scheme that had produced the maximal (FT) for the individual. Another 3-week training period, using the loading scheme that resulted in the lowest (FT) was also completed. The results of the study revealed that when training using the maximal FT loading scheme, there was a 3% increase in strength as opposed to a 3% decrease in strength using the minimal FT loading scheme. Therefore, resistance training that elicits the optimal individual FT response resulted in the best improvement in muscle size and strength. Based on the plethora of research linking circulating T and FT with skeletal muscle hypertrophy (Ahtiainen et al., 2003; Beaven et al., 2008; Bhasin et al., 1996; Häkkinen et al., 1988; Sinha-Hikim et al., 2002), it seems reasonable to conclude that exercising with an elevated circulating T or FT concentration leads to skeletal muscle hypertrophy and strength.

A proposed mechanism responsible for the observed increases in hypertrophy and strength of skeletal muscle as a result of exogenous or endogenous increases in systemic T is the observed augmented myonuclear to fiber ratio and satellite cell number (Sinha-Hikim et al., 2002). Increases in myonuclear number to fiber ratio as a result of proliferated satellite cells is a mechanism responsible for hypertrophy of myofibrils (Sinha-Hikim et al., 2002). Additionally, accompanying changes in body fat can be explained by the differentiation of mesenchymal multipotent cells to myogenic and not adipogenic cells as a result of T binding with the androgenic receptor in the skeletal muscle (Hoffman et al., 2009). Differentiation of the mesenchymal multipotent cells occurs when FT is bound to the androgenic receptor  $\beta$ -catenin and subsequently forms a larger complex with T-cell factor 4 in the nucleus. The T-cell factor 4 complex then

promotes myogenic differentiation through its influence on Wnt-regulated genes in the muscle cell's deoxyribonucleic acid (Hoffman et al., 2009; Zhou et al., 1994).

#### Indirect Effects of Testosterone on Skeletal Muscle

Indirect effects of T on muscle hypertrophy may be mediated by circulating IGF-1 concentrations that are also augmented with higher doses of injected T (Bhasin et al., 2001; Sinha-Hikim et al., 2002), although the exact linkage between the two hormones is not clear. Intramuscular IGF-1 and IGF-1 receptors may be regulated by systemic T concentrations (Bamman et al., 2008). Another indirect effect of T occurs when T is bound to the cytoplasmic androgen receptor which attenuates the inhibitory action of myostatin on muscle hypertrophy (Hoffman et al., 2009). Furthermore, the anabolic action of circulating T to increase skeletal muscle size is also mediated through either blocking (Mayer & Rosen, 1975), or down regulating glucocorticoid receptors, reducing the action of the catabolic hormone cortisol (Hickson et al., 1990). Testosterone's receptor has also been observed to be up-regulated with resistance training in the presence of adequate nutrition and may provide a better opportunity for T binding (Bamman et al., 2001; Kadi et al., 2000; Spiering et al., 2009).

#### Resistance Exercise and its Influence on the Androgen Receptor in the Presence of Testosterone

While the circulating T concentration is important and frequently measured when examining the influence of T on hypertrophy, there is another part to the puzzle that is just beginning to be investigated. As with any hormone, the receptor density in the target



tissue is important in determining the effect of the hormone on the target tissue.

Testosterone's receptor, found in skeletal muscle, is the androgen receptor (AR).

A significant reduction in AR density has been shown in men 60 to 70 minutes post resistance training (Ratamess et al., 2005; Vingren et al., 2009) possibly from the binding of T to the AR or a high physiological stress resulting in catabolism (Ratamess et al., 2005). However, Spiering et al. (2009), Bamman et al. (2008), and Willoughby et al., (2004) found AR density was significantly increased at 180 mins post resistance training and after 2-3 days of resistance training, respectively. Ahtiainen et al., (2009) also found that resting AR density was increased one fold after 21 weeks of strength training. Additionally, trained powerlifters on steroids had a significantly higher density of ARs in the trapezius but not the vastus lateralis as compared with non-steroid using powerlifters (Kadi et al., 2000). Nonsteroid using powerlifters had more ARs than untrained individuals (Kadi et al., 2000). Therefore, while the circulating T concentration is important, the AR density seems to be also modified as a result of a REB and training background.

One study that has examined the AR response to a REB was performed by Spiering and colleagues (2009). The authors speculate that T reduces AR density catabolism as the result of training because of the doubling of AR concentrations in skeletal muscle in the presence of increases in circulating T. The maintenance of AR receptor density could lead to a greater potential for T to bind. Such a relationship between T concentration and the upregulation of its receptor highlights the possible link between circulating T and its effect on exercising muscle exposed to increases in T concentrations.

The experimental group in the study by Spiering and colleagues benefited from an added upper body resistance exercise volume before the lower body resistance exercise. The lower body resistance exercise and not the upper body resistance exercise was performed by the control group. The authors suggested that the addition of the upper body exercise in the experimental group served a ‘priming’ function by resulting in a greater T concentration which in turn resulted in a significant increase in AR density in the vasus lateralis 180 minutes post training bout. The increase in the AR density with the additional exercise volume and resulting higher T concentrations indicates that the more work performed during a resistance bout the greater the AR receptor density. Interestingly, based on the results of Spiering and colleagues, limbs that are ipsilateral to the exercising limbs still benefit in an improved AR density up to 180 minutes post-REB as long as the total T concentration is elevated.

Clearly, changes in the skeletal muscle AR density are important in determining the effects of T on skeletal muscle hypertrophy. However, measuring AR density in the skeletal muscle is difficult because of the need for advanced biopsy techniques and equipment. Fortunately AR density may be dependent on total T concentrations and thus a REB resulting in a higher T concentration should produce a greater AR density (Spiering et al., 2009). Therefore, when comparing the T concentrations resulting from different REBs it may be possible to infer that increases in the AR density in the skeletal muscle occurs with increases in T concentration.

### Cortisol: Background

Cortisol is responsible for the breakdown of muscle protein into its amino acid bases to be used for gluconeogenesis in the liver or proteogenesis in a variety of other

tissues. Therefore, C can be a potent hormone for limiting the development of skeletal muscle hypertrophy and strength. Cortisol is classified as a glucocorticoid because of its synthesis in the cortex of adrenal glands and the role C plays in the maintenance of normal blood glucose.

Cortisol production is initiated as a result of psychological or physiological stress such as anticipation of and during a REB. An initial step in the production of C is the hypothalamic production and secretion of corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) into the hypophyseal-portal circulation. Corticotrophin releasing hormone and AVP synergistically stimulate the corticotropes of the anterior pituitary to produce and secrete adrenocorticotrophic hormone (ACTH) into the systemic circulation. Once circulating ACTH binds to the corticotrophin receptors of the adrenal cortex, the adrenals produce and release C into the systemic circulation (Kramer & Rogol, 2005).

Cortisol is regulated by a negative feedback loop wherein elevated C concentrations reduce either the release of CRH and AVP at the hypothalamus or ACTH at the anterior pituitary (Kraemer & Rogol, 2005). In addition to the direct effects of exercise stress, C concentrations are also affected indirectly by exercise-mediated changes of circulating glucose (Kraemer & Rogol, 2005). Therefore, after a short, high intensity or long, low intensity bout of exercise when blood glucose levels fall, concentrations of C increase and contribute to the maintenance of blood glucose. The C-mediated elevation in glucose is through C's proteolytic action in liberating amino acids and C's lipolytic action on adipose cells resulting in liberated glycerol. As previously mentioned, both the circulating amino acids and glycerol are taken back to the liver

where they are metabolized into glucose through gluconeogenesis, with the subsequent release of glucose circulation.

### Total Versus Free Cortisol

Like T, C is a nonpolar steroid hormone derived from a cholesterol base. Therefore around 90% of C in the circulation is bound to either cortisol-binding globulin (CBG) or albumin, with the remaining 10% circulating as free or unbound C (Gayard et al., 1996; Kraemer & Ratamas, 2005). However, interperson variability in CBG levels has been shown (Dhillon et al., 2002) and thus might influence free C concentration. Additionally, CBG like SHBG may have access to skeletal muscle through a plasma cell membrane receptor-mediated endocytotic process (Breuner & Orchinik, 2002) which suggests the total concentration of C has greater physiological meaning than just free C concentrations (Hyrb et al., 1987; Strel'Chyonok & Avvakumov, 1991). Concentrations of free and total C run in parallel, so total C should also follow the same pattern as free C after a bout of exercise (Heyns & Coolens, 1988). Therefore, not surprisingly the majority of the resistance training research using C as a marker of protein catabolism has used total cortisol (Kraemer & Ratamess, 2005).

### The Effect of Cortisol on Skeletal Muscle

Either a reduction in the sensitivity of skeletal muscle to C or and a reduction in the circulating concentrations of C may contribute to increases in muscle size and strength. The possible mechanism of the muscles' resistance to the affects of C could be through a desensitivity of muscle to cortisol via the down-regulation of glucocorticoid receptors (Kraemer & Ratamas, 2005). A down-regulation of glucocorticoid receptors

can occur as a result of hypercortisolism produced from frequent, intensive training (Kraemer & Rogol, 2005). Along with a down-regulation in the glucocorticoid receptor, an actual reduction in circulating C could occur from an increase in the concentrations of opioids and analgesic  $\beta$ -endorphins. With training,  $\beta$ -endorphin competes with ACTH for their common precursor molecule proopiomelanocortin polypeptide (Kraemer & Rogol, 2005). The resulting reductions in ACTH production reduce C production and release from the adrenal cortex. If the muscle sensitivity to C is reduced and C concentrations are lowered, the catabolic effect on skeletal muscle is diminished.

Interestingly, after 9 weeks of training, post-REB C concentrations start to decline which corresponds to the time point at which changes in muscle cross sectional area start to appear. A 9-week resistance training program with trained males resulted in a moderate inverse correlation ( $r^2 = -.46$ ) between the change in type IIX cross sectional area and changes in resting C, suggesting the possible importance of reduced C concentrations on muscle hypertrophy (Staron et al., 1994). Cortisol has also been shown to inhibit nocturnal T production which would also support the notion that C concentrations must be considered when determining the effect of T on skeletal muscle hypertrophy (Doerr & Pirke, 1976).

#### Testosterone and Cortisol Kinetics in Response to Training

The increase in T during and up to 2 hrs after exercise training and a reduction in T between training bouts have encouraged the use of T as a measure of training intensity, training stress and overtraining (Bosco et al., 2000; Fry et al., 1992; Raastad et al., 2001). Reductions in T over the course of a heavy resistance training program are frequently seen at the point in the training program where the training volume-load is the highest in

trained individuals (Häkkinen et al., 1988; Raastad et al., 2001). Performance is improved when T concentrations are restored back to normal or greater than normal concentrations after a period of recovery, emphasizing the importance of have a period of the training cycle devoted to restoring T concentrations to baseline or greater levels (Häkkinen et al., 1988).

Circulating T and C were elevated (30.4% and 12%, respectively) after an aerobic exercise bout performed pre- and postinvolvement in a 5-week individual based progressive training program emphasizing a combination of resistance, speed, anaerobic and aerobic training (Pitkänen et al., 2002). During the 5-week period, serum protein values were significantly reduced possibly linking protein utilization during periods of increase T concentrations (Pitkänen et al., 2002; Sallinen et al., 2004). In untrained males, resting T concentrations rise over 8 weeks of resistance training as opposed to the decrease seen with trained males (Kraemer et al., 1998). Conversely, C concentrations decrease as an individual becomes trained (Hansen et al., 2001) which again indicates the anabolic environment is improved over the course of training.

In support of the importance of the anabolic environment over the course of heavy training, changes in T and C were recorded during a collegiate soccer season. An upward trend in T concentrations was observed over the six time points starting from the preseason camp (Kraemer et al., 2004). Performance values such as the 20m sprint and the vertical jump showed a general decrease in performance (significantly reduced at time point 5) over the six time points taken during the season. At time points where T values increased, performance in the 20m sprint and vertical also improved. However, this

relationship between T and performance is only an observation and was not statistically analyzed.

Cortisol remained unchanged throughout the season except interestingly at the time point preceding the significant decline in vertical jump performance, cortisol increased (Kraemer et al, 2004). The importance of maintaining high concentrations of T and low concentrations of C may be critical to maintain or improve athletic performance. It is clear that a relationship exists between T concentrations and the current training load placed on the body and current training status of the individual, respectively. Similarly, C seems to fluctuate with training load and training status.

#### Nonexercise Factors Affecting Testosterone and Cortisol Concentrations

Testosterone and C concentrations are affected by a myriad of factors besides exercise. Those factors include circadian rhythm, body composition, diet, age, sex, training status, hydration and changes in plasma volume. Each factor can alter the circulating concentrations of T and C and therefore must be controlled for in order to clearly identify changes in T and C from an exercise bout or training.

#### Diurnal Fluctuations

Testosterone concentrations are individualized in regard to a diurnal rhythm of pulsatile T release. Spratt et al., (1988) showed that over the course of a day, resting T declines in some participants but not others. Other research has shown a clear decline in T throughout the day (Bremner et al., 1983). Interestingly, Spratt et al. (1988) also observed that in half the men assessed, resting T concentrations dropped below the lower

bound of 'normal' T concentrations at some point during the 24 hrs of the study. Like T, C is also under the influence of diurnal fluctuations. Cortisol declines throughout the day but is influenced by the timing of both exercise (Brandenberger & Follenius, 1975) and pre- or postexercise meals (Brandenberg et al., 1982).

In an attempt to account for the potential variations in the diurnal cycle some researchers have performed a control day and matched the assessments times to the experimental protocol (Häkkinen et al., 1993; Schwab et al., 1993). Although not statistically analyzed, T concentrations did show a declining trend during the control day over the course of 16 minutes (19.39 to 16.28nmol/L) (Schwab et al., 1993). A significant decline over 12 hrs was observed on a control day with T values the highest at 8:00 (~28nmol/L) and the lowest at 20:00 (~22nmol/L) (Häkkinen et al., 1993). The daily decline in T emphasizes the importance of controlling sampling times for the accurate assessment of the change in T values over the course of a training bout or day.

### Body Fat

Testosterone values in males can be significantly depressed if the individual is obese because body fat aromatizes T to estradiol through the enzyme aromatase (Vettor et al., 1997). Vettor and colleagues, (1997) observed a moderate negative correlation between body weight ( $r = -.54$ ) and basal T and between body mass index and basal T ( $r = -.62$ ). When grouping individuals according to body mass index the obese group ( $M = 32\text{kg/m}^2$ ) had significantly less circulating FT ( $18.6 \pm 1.3\text{ng/L}$  vs  $23.3 \pm 1.4\text{ng/L}$ ;  $p < .01$ ) as compared to the lean group ( $M = 21.6\text{kg/m}^2$ ). Possible mechanisms for the negative relationship between body composition and body mass index could be from T decreasing the expression of the *ob* gene in adipocytes which trigger the production of leptin (Vettor



et al., 1997). Regardless of the cause, the point is obese individuals can present confounding T concentrations in a research study and therefore obese participants should be excluded from investigations examining the T response in the athletic and nonobese and nonoverweight populations.

### Diet

Strong correlations have been found in young, strength trained males between resting values of T and percentage of calories from protein ( $r = -.71$  and  $-.77$ ), g/kg of protein ( $r = -.68$ ), percentage of calories from fat ( $r = .72$ ), g/kg of fat ( $r = .65$ ), g/1000kcal per day of saturated fat ( $r = .77$ ), g/kg of monounsaturated fat ( $r = .90$ ) and g/1000kcal per day of mono unsaturated fat ( $r = .79$ ) (Sallinen et al., 2004; Volek et al., 1997). Polyunsaturated fat did not correlate strongly with T (Sallinen et al., Volek et al.). Additionally, strong correlations were also seen between the change in T after a forced repetition resistance training bout and the percentage of calories from protein ( $r = -.81$ ), g/kg of protein intake ( $r = -.86$ ), the percentage of calories from fat ( $r = .85$ ), and g/kg of fat intake ( $r = .72$ ) (Sallinen et al., 2004). The strong correlations with fat illustrate the potential importance of providing dietary cholesterol for the construction of testosterone in the testes. Ingested dietary cholesterol has also shown to be moderately correlated ( $r = .53$ ) with resting T concentrations, supporting the link between cholesterol and T (Volek et al., 1997). Along with the strong correlation with dietary fat, a high carbohydrate diet produces significantly higher T concentrations when compared to a high protein diet (Anderson et al., 1987).

While serum protein is negatively correlated with T after an acute bout of resistance exercise, during a 4-week, high volume, high intensity resistance training

program accompanied by amino acid supplementation, a significantly higher T value was observed for the first 3 weeks of the training program (Kraemer, Ratamas et al., 2006). However, testosterone values were not significantly different after 4 weeks. The impact of a set dose of an amino acid supplement on T may be effective only for the first 3 weeks of a high volume, high intensity resistance training program (Kraemer, Ratamas et al., 2006). If T is important in hypertrophy, a change in the amino acid dose or configuration may be needed to see a continued elevation in T with more than 3 weeks of training.

Cortisol is also influenced by dietary factors, since a high protein diet has been shown to significantly reduce C concentrations as compared to a high carbohydrate diet (Anderson et al., 1987). Therefore, with the influence of diet on concentrations of T and C, any study design should include a manipulation check to ensure that changes in T and C as a result of exercise are not confounded by changes in diet over the course of the study.

### Age

Age is another possible confounding variable that can influence resting T and C concentrations and the response of T and C to a bout of exercise. However, no differences were seen in resting and T and C responses after a resistance training bout of identical volume between young ( $23 \pm 1$  yrs) and old ( $69 \pm 5$  yrs) men (Smilios et al., 2007). Similarly there was not an increase in T between middle-aged (age = 46 yrs) and older (age = 64 yrs) men over 16 weeks of training (Izquierdo et al., 2001). A significant decrease in C was observed in the older men but not the middle-aged men over the 16 weeks (Izquierdo et al., 2001). Strength assessments of the upper and lower body

revealed that the middle-aged men experienced greater increases in strength than the older men (Häkkinen et al., 1998; Izquierdo et al., 2001). Although not significant, there was an increase in T over 16 weeks from baseline in the middle-aged men (18.6nmol/L to 19.4nmol/L) and not the older men (18.8nmol/L to 17.8nmol/L) that could have contributed to the improvements in strength (Izquierdo et al., 2001). The initial T values measured 4 weeks before the study showed a greater absolute difference of 1.7nmol/L in the middle-aged versus older men and thus could be the reason for the greater improvements in strength in the middle-aged men (Häkkinen et al., 1998). The ability to perform more work, as measured by a significantly greater increase in circulating lactate concentrations, may also be a factor in younger men producing more T after resistance training than older men (Häkkinen et al., 1998).

A positive correlation ( $r = .37$ ) has been reported when comparing 24-hour total circulating cortisol and age (Purnell et al., 2004). However, body composition was not associated with free C concentrations (Purnell et al., 2004). The results of the research literature emphasize the importance of obtaining a group of participants of similar age (college-aged, etc.) when studying the T and C response to resistance training.

### Training Status

Training status is an important factor when considering the T response to resistance training. Seven sedentary, 8 endurance trained and 7 resistance trained males performed both an aerobic bout and separate resistance exercise bouts of equal volume (kcal) (Tremblay et al., 2004). For the aerobic training group, resistance training produced significant reductions in T as compared to a 40-min run. The T response was specific to the training status of the athlete and the mode of exercise, for example the

resistance trained males produced significant changes in T after resistance training while the aerobically trained group did not. The sedentary group experienced significant changes in T with both resistance and aerobic training. Similarly, strength trained males produced a greater T concentration, postresistance training bout, than untrained participants (Ahtianinen et al., 2004). Therefore, trained participants and in particular resistance trained participants should be selected if acute changes in T are to be measured with a resistance training protocol.

Cortisol concentrations show a similar response between sedentary, endurance trained and resistance trained individuals (Tremblay et al., 2004). However, the magnitude of increase in C after resistance training, although significant for all groups, was greater in the sedentary and resistance trained groups. The elevation in C indicates that both groups were working with intensities of exercise that significantly challenged the individuals leading to a greater stress and C concentrations.

Strength trained males also demonstrated the same recovery C concentrations as untrained males but when coupled with a greater increase in T, strength trained males produce a greater anabolic milieu (Ahtiainen et al., 2004). Trained body builders, accustomed to high volume resistance training, when compared with powerlifters, accustomed to low volume resistance training, did not show a significant difference in C concentrations post resistance exercise bout (Kreamer et al., 1987). Therefore, it is the resistance training status of the individual and not only the type of resistance training used during training that affects post-REB C concentrations. A drop in post-REB C with resistance training may be as a result of the conversion of fast fatigable muscle fibers

(type IIx) to intermediate fibers that is known to occur with resistance training (Staron et al., 1994).

In contrast to evidence that shows reductions in C in resistance trained males (Hansen et al., 2001), untrained males after 8 weeks of resistance training and elite weightlifters after 6 weeks of resistance training showed significant elevation in basal C concentrations (11.1% and ~10%, respectively) (Santilla et al., 2009). Therefore, at the extremes of resistance training status, the untrained and elite resistance trained may produce increases in C while the majority of strength trainees will experience decreases in C over the course of a training program (Häkkinen et al., 1985). Therefore, when constructing a study using resistance exercise it is important to have a homogenous group of individuals with a similar training background in order to ensure that any changes in the C response are not due to training background.

### Hydration

Hydration also has a significant effect on T and C concentrations immediately after a bout of resistance exercise (Judelson et al., 2008; Kraemer, Spiering et al., 2006). When compared to euhydration, reductions of 2.5% and 5% bodyweight through dehydration produces a significant decrease in T (10.8%, NS and 16.8%,  $p < .05$ , respectively) (Judelson et al., 2008). A significant increase in C at every 15-min interval from the end of the resistance training bout to 60 minutes post was also seen when the dehydrated conditions were compared to the euhydrated condition (Judelson et al., 2008). Individuals must be euhydrated to maintain homeostatic concentrations of T and C.

### Changes in Plasma Volume

In contrast to the view that T and C production increase with resistance exercise, a decrease in plasma volume during and post-REB (~14%) (Kraemer et al., 1993) also is responsible for the post-REB elevations in T and C concentrations (McCall et al., 1999). Significant elevations in T during and after a REB are seen when not corrected for the shift in plasma volume (Kraemer, Kilgore et al., 1992; McCall et al., 1999); however, when plasma volume changes were accounted for, the significant changes in T disappeared (Kraemer, Kilgore et al., 1992; McCall et al., 1999), highlighting the contribution of the shift in plasma volume to changes in concentrations of T. While decreases in plasma volume appear to contribute to elevations in circulating T values, not all resistance training studies have shown a nonsignificant plasma volume corrected T response during and post-REB (Cumming et al., 1987; Schwab et al., 1993). Additionally, a significant increase in C immediately post-REB has been reported for both corrected and uncorrected concentrations of C (McCall et al., 1999). Therefore, both plasma volume corrected and uncorrected values are important in assessing the T and C responses to resistance training.

### The Effect of Resistance Training Program Variables on Testosterone and Cortisol Concentrations

A resistance training bout designed to increase circulating T values has been extensively researched (Kraemer et al., 1990, 2006; Yarrow et al., 2007). Total volume (total repetitions) (Gotshalk et al., 1997; Häkkinen et al., 1993; Kraemer et al., 1990; Ratamess et al., 2005), volume-load (load x total repetitions) (Gotshalk et al., 1997; Häkkinen et al., 1993; Kraemer et al., 1990), intensity (% of 1-repetition maximum)

(Häkkinen et al., 1993; Kraemer et al., 1990; Schwab et al., 1993), rest period (Ahtiainen et al., 2009; Buresh et al., 2009; Goto et al., 2005; Kraemer et al., 1990), muscle action (concentric vs. eccentric) (Durand et al., 2003; Kraemer et al. 2006; Yarrow et al., 2007), maximal versus forced repetitions (Ahtiainen, Pakarinen, Kraemer et al., 2003) and mode of training (isokinetic, isometric, dynamic etc) have all been analyzed.

### Intensity

The optimal intensity to elicit T varies between individuals (Beaven et al., 2008). A comparison of low intensity (60-65% of 6RM) and moderate intensity (90-95% of 6RM) resistance training showed no significant differences over four sets of squats although T was significantly elevated from baseline for both intensities (Schwab et al., 1993). A nonsignificant difference in absolute T area under the curve was also observed from pre-REB to 120 mins post-REB when a 5-RM (~380ng/dl) vs 10-RM (~180ng/dl) intensity was used, although the absolute area under the curve for T was greater for the 5-RM loading (Kraemer et al., 1990). A similar study, using a high volume (15 repetitions), low intensity (60% of 1-RM) and high intensity (90% of 1-RM), low volume (5 repetitions) scheme also showed no difference in the T area under the curve (Hoffman et al., 2003). However, two studies observed greater recovery T values after a higher volume 10-RM scheme as compared to a 1-RM and 5-RM scheme at recovery time points immediately post-REB (Häkkinen et al., 1993) to 30 minutes post-REB (Kraemer et al., 1991). Therefore, a REB of a moderate intensity (65-75% of 1-repetition maximum or 10-repetition maximum) is the most effective training intensity to raise circulating T concentrations.

### Volume, Rest Period Length and Repetitions to Failure

Studies comparing different volume-loads arranged with different sets and repetition schemes with a focus on power, strength or hypertrophy development have shown that hypertrophy loading schemes emphasizing large volumes (sets x repetitions), short ( $\leq 2$  min) to no rest periods, and repetitions to failure produce the greatest increases in acute post-REB T concentrations (15-89% greater from baseline) (Ahtiainen et al., 2004, 2005; Buresh et al., 2009; Crewther et al., 2008; Goto et al., 2005; Häkkinen et al., 1993; Jürimäe et al., 1990). While elevations in T were the greatest with this high volume scheme, C concentrations postexercise were also the greatest with a high volume scheme (Ahtiainen, Pakarinen, Alen et al., 2003; Buresh et al., 2009; Crewther et al., 2008; Häkkinen et al., 1993; Ratamess et al., 2005; Smilios et al., 2003).

When examining the affect of volume on T, Ratamess et al. (2005) observed a significantly greater T response immediately to 45 mins post after three sets of resistance training compared to only one set. Additionally, the recovery kinetics of T indicate a greater anabolic response after a high volume scheme ( $\geq 30$  reps) compared to a low volume scheme ( $< 30$ ). The optimal volume needed to significantly elevate T may be  $\sim 40$  repetitions according to the results of Smilios et al. (2003) who compared varying volumes of resistance training on T concentrations.

In another comparison of the affect of different training volumes on circulating T, Kraemer et al. (1990) found that there was not a significant difference in T area under the curve when analyzing differing volume schemes (3 sets of 10 repetitions using 1 min rest vs. 5 sets of 3-5 repetitions using 3 minutes rest) (Kraemer et al., 1990). As mentioned, the 5-RM, lower volume training routine produced greater increases in T concentrations



than the 10-RM high volume training (~380ng/dl vs. ~180ng/dl) although the difference was not significant (Kraemer et al., 1990). However, when one set of 10 repetitions was compared with three sets of 10 repetitions the higher volume from the three sets of 10 groups produced significantly greater T and C concentrations at immediately post, 5 mins, 15 mins and 30 mins post bout (Gotshalk et al., 1997; Ratamess et al., 2005). The discrepancy between the results using low or high volume has been interpreted to suggest that there is probably an individual response to training load and volume with T production (Beaven et al., 2008).

The C response is also sensitive to changes in volume (Ratamess et al., 2005; Smilios et al., 2003), although some evidence does implicate intensity as another contributing factor in the C response to an REB (Charro et al., 2010). The C response is potentially greater if the resistance exercise volume and or volume-load is greater. However, C may also be sensitive to a threshold of resistance training intensity, that when reached increases the T response even if the exercise volume is low. Conversely, a volume threshold may exist when the intensity of the REB is low. However, REBs using a volume of 25-40 repetitions per exercise and a resistance training intensity of ~70% of one-repetition maximum appear to be above both the volume and intensity thresholds (Kraemer et al., 1990; Smilios et al., 2003)

When assessing the T response from an acute bout of resistance exercise, a shorter rest period ( $\leq 1$ mins) is effective in elevating T post-REB (Buresh et al., 2009; Kraemer et al., 1990). Buresh et al. (2009) found T and C concentrations were significantly greater post-REB when using a 1 min vs. 2.5 min rest between resistance exercises. Ahtiainen et al. (2005) found that a rest period of 2 mins significantly elevated T and C immediately

post-REB. Based on the research a rest period  $<2$  mins will result in an elevation in T, important for studies desiring an increase in T as a result of resistance exercise.

Another resistance exercise variable is the performance of the last repetitions of a set. The first of two primary approaches to the last repetitions of a set is to limit the number of repetitions performed to the prescribed number of repetitions. The prescribed number of repetitions is set with a load great enough to not allow any additional repetitions, thus the name maximal repetitions. The second way to treat the last repetitions in a set is by doing as many repetitions as possible per set regardless of whether the repetitions performed exceed the repetitions prescribed. Completing the final repetitions in this manner is called repetitions to failure. Performing either maximal repetitions or repetitions to failure during a set have been equally effective in elevating T above baseline (Ahtiainen, Pakarinen, Kraemer et al., 2003). Although there is evidence that performing repetitions to failure may be more effective in elevating T (Häkkinen et al., 1993). Additionally, C responses appear to be significantly greater using repetitions to failure per set (Ahtiainen, Pakarinen, Kraemer et al., 2003; Häkkinen et al., 1993). Therefore, using repetitions to failure should result in an increase in both T and C and should be used in studies desiring significant elevations in T as a result of resistance exercise despite a concurrent increase in C.

### Resistance Training Modality

Muscle action has been analyzed with respect to the possibility that eccentric muscle contractions may be more beneficial than traditional concentric/eccentric dynamic training in elevating T (Kraemer et al., 2006). However, a significant difference in the T response after eccentric training at 100% of 1-RM and traditional concentric/eccentric

training using 52.5% of 1-RM was not observed (Yarrow et al., 2007, 2008). While total volume was different between the eccentric and traditional concentric/eccentric groups there was not a significant difference in T production between the groups (Yarrow et al., 2007, 2008).

The concentric portion of a dynamic resistance exercise movement has been shown to be the most important in increasing T concentrations (Durand et al., 2003). Traditional concentric/eccentric dynamic training (Kraemer, Hollander et al., 2006) is equally effective as eccentric only training in increasing T concentrations (Yarrow et al., 2007, 2008). Based on the current evidence, the use of dynamic resistance exercise utilizing both concentric and eccentric muscle concentration is consistently the most effect mode of resistance exercise to increase circulating T concentrations.

#### Change in Testosterone and Cortisol Concentrations During and After a Bout of Resistance Exercise

Testosterone values significantly rise over the course of an REB (French et al., 2007) leading to a peak in T which can occur from immediately to 5 min (Kraemer et al., 1991; Kraemer, Fry et al., 1992; Sallinen et al., 2004; Vingren et al., 2009; Yarrow et al., 2008) to 30 mins post-REB (Yarrow et al., 2007). In some instances there is not a significant peak in T (West, Burd, Staples et al., 2010). For those studies where a peak in T does not occur, the muscle mass utilized was probably too small (West, Burd, Staples et al., 2010) or the volume of work was probably too small (West, Burd, Staples et al., 2010). For those studies that do see a peak in T, T values decline after 30 mins, typically to below baseline values for up to 60 to 120 mins post-REB (Häkkinen et al., 1995; Vingren et al., 2009; Yarrow et al., 2007, 2008). Then T values trend upwards for up to

13 hours postexercise (Nindl et al., 2001). Twenty-four to forty-eight hours post-REB, T values recover back to baseline concentrations (Jürimäe et al., 1990; McCall et al., 1999).

In a study using squats of two intensities, one low (60-65% of 6RM) and one moderate (90-95% of 6RM), circulating T concentrations were not significantly elevated until the fourth set of the exercise bout for both intensities (Schwab et al., 1993). While the trends between the two loading schemes were not analyzed, the relationship from baseline to postexercise was linear for both groups, although the slope of the line for both exercise intensities appeared to be different (Schwab et al., 1993). The results of the study by Schwab and colleagues support the contention that the intensity of an REB is not an important factor in elevating T during a REB.

Cortisol responses during aerobic exercise are varied as shown by Viru and Smirnova, (1992). The authors observed two patterns of the response of C during a submaximal 120-min continuous bout of aerobic exercise. The first pattern consisted of an increase in C up to ~30 mins followed by a decrease or maintenance in C and then a second linear climb or maintenance in C toward the end of the 120-minute protocol. The second pattern was characterized by a linear increase in C from the beginning of exercise until the end of the 120-min bout. Training status appeared to have little effect on the pattern of C, emphasizing the interperson variability in C response during exercise. The individual response curves do illustrate some individual variability in the increase in C during exercise, perhaps reflecting differences in individual glycogen reserves, since a decrease in muscle glycogen would necessitate the need for other sources of fuel, including glucose derived from C facilitated gluconeogenesis. During recovery after an REB C has been observed to peak 30-45 mins post REB, then C concentrations start to

return to baseline at 60 mins post-REB (Crewther et al., 2008; Nindl et al., 2001).

Additionally, C concentrations are elevated overnight for a groups completing a REB when compared to a control group (Nindl et al., 2002).

It is a possibility that the variations in the C response were due to variations in the circadian rhythm of C (Purnell et al., 2004; Thuma et al., 1995). Although the time of day was standardized, the initiation of the bouts of exercise in the study by Viru and Smirnova (1992) were 2 hrs apart which may have been the source of measurement error. Performing an REB at the same time of day during a study with repeated bouts is critical to ensure that circadian rhythms do not confound the C values observed between bouts.

#### Testosterone and Cortisol Responses to Body Segment Specific Resistance Exercises

The comparison of the T response to segment specific exercise (i.e., upper vs. lower body) has only been attempted by a few researchers (Häkkinen et al., 1998; Madarama et al., 2010; Volek et al., 1997). Volek et al. (1997) compared a lower body power exercise, the squat jump, to an upper body resistance exercise, the bench press. A statistical comparison was not made between the change in T after the squat jump and bench press. However, a visual inspection of the data does indicate there is a greater increase in T from pre to post measurements in the squat jump (~15%) when compared with the bench press (~7%) (Volek et al., 1997). Häkkinen et al. (1998) analyzed the T response to a lower and upper body isometric exercise (squat vs. bench press). Häkkinen and colleagues found that both a lower and upper body bout performed separately, along with a bout using the combination of the lower and upper body exercises produced a significant increase in T in young men. Only one study has directly compared the T

response after an upper and lower body resistance exercise. Madarama et al. (2010) used restricted blood flow training and found a nonsignificant difference in post bout T when an upper and lower body REB were compared. However, C remained elevated up to 15 minutes post bout for both the upper and lower body REBs and C remained significantly elevated up to 30 mins post the lower body bout (Madarama et al., 2010). No acute study has directly compared the pre to post change in T after a separate REB using only a typical dynamic upper body and dynamic lower body resistance exercise.

A chronic training study measuring the T and C response to two training schemes, one using 8 sets of only dynamic biceps curling and the other using dynamic biceps curling with the addition of 8 sets of leg press has been performed (Hansen et al., 2001). Both the biceps curling and leg pressing followed a descending load scheme wherein the load was reduced after each set in order to achieve 8-12 repetitions per set. The scheme was designed to elicit muscle hypertrophy because of its high volume using only a constant load for each set. The results from the Hansen et al. (2001) investigation did not show any significant differences in the acute T response immediately post and at 15, 30 and 60 minutes after the first and final exercise bout of the 9-week study. Although the results were not statistically different, the immediately post measurement, where T values are the greatest, displayed a difference of ~115ng/dl between the upper body only and upper body plus lower body training groups. The authors speculated that the significantly greater increase in the trained arm from the upper body plus lower body group as compared to the upper arm group alone is from the greater increase in acute T from the addition of the lower body sets.

In contrast to the study by Hansen et al. (2001), West, Burd, Staples et al. (2010) attempted a similar training study but over the course of 15 weeks instead of 9 weeks. Additionally, West, Burd, Staples and colleagues trained the biceps but this time with both arms training under differing concentrations of T. One arm was trained under low T concentrations (arm only training) and the other arm was trained in the presence of high T generated from the addition of a leg press exercise before arm exercise. There was not a significant difference between the changes in cross sectional area and arm strength based on training condition.

The importance of performing resistance training in an anabolic milieu was also analyzed by Wilkinson et al. (2006). The study design consisted of each participant exercising only one leg. The cross sectional area of both the trained and untrained leg were measured in order to determine if exposing nonexercising limbs to high T concentrations improves muscle cross sectional area. Changes in cross sectional area in the trained ( $5.4 \pm 0.9\%$ ) but not the untrained leg ( $0 \pm 0.5\%$ ) were observed, supporting the point that endogenous increases in T do not increase muscle cross sectional area independent of training. However, the training stimulus was not great enough with single leg training to significantly elevate circulating T values post resistance training bout. Additionally, the location where the CT scan occurred in order to determine changes in muscle cross sectional area was fixed. The muscle may have hypertrophied proximal or distal to the point analyzed. Even if an increase in T as a result of training does not increase muscle cross sectional area in nonexercising limbs, the effect of T on exercising limbs probably results in muscle hypertrophy (Ahtiainen et al., 2009; Beaven et al., 2008; Hansen et al., 2001; Sinha-Hikiam et al., 2002; Staron et al., 1994).

In contrast to T, C levels were not elevated after either an upper body or lower body REB (Häkkinen et al., 1998) probably because of the loading scheme and the use of isometric contraction. In the 9-week training study by Hansen et al. (2001) the combined upper body and lower body REBs did produce a significantly greater C response than the upper body REB alone. However, the difference in C between the combined and upper body alone group was only observed at the beginning and not after the 9-week training protocol. The results indicate that the increase in the total amount of work due to the addition of lower body exercise to the upper body exercise may lead to a period of higher post-REB C concentrations. However, the higher C concentrations gradually decline over 9 weeks of training, even as volume-load is adjusted with training induced strength gains warranted (Hansen et al., 2001).

### Resistance Exercise, Lactate Concentrations, and Session Rating of Perceived Exertion

#### Lactate Responses to Resistance Training

A common measure of the intensity of a REB is postexercise systemic lactate concentration (Vingren et al., 2008). Lactate concentration has been shown to peak immediately after an REB (Häkkinen et al., 1993; Kraemer, Kilgore et al., 1992; Kraemer et al., 1998; Vingren et al., 2008) and decrease after 60 mins posteccentric resistance exercise bout ( $>5\text{mmol/L}$  to  $<1\text{mmol/L}$ ). Lactate concentration is still elevated ( $>1\text{mmol/L}$ ) at 60 mins post REB with dynamic concentric and eccentric resistance exercise (Ratamess et al., 2005; Vingren et al., 2008; Yarrow et al., 2008). Kraemer, Kilgore and colleagues (1992) observed that lactate concentrations finally declined 95 minutes post REB.



The REB scheme also affects lactate concentration. Post REB lactate concentrations have been reported as high as 18mmol/L after 10 exercises of 3 sets of 10 repetitions utilizing 30-60s rest periods (Kraemer et al., 1987) and 8.61mmol/L after 3 sets of 10 repetitions utilizing 8 exercises and 4.39mmol/L after 5 sets of 3-5 repetitions utilizing 2 min rest periods during 8 exercises (Kraemer, Kilgore et al., 1992). Significant elevations in lactate occur after 3 sets of 10 repetitions as compared to 1 set of 10 repetitions (Gotshalk et al., 1997; Ratamess et al., 2005). Therefore, lactate concentrations are significantly higher after a typical hypertrophy resistance training scheme of high volume and short rest period when compared to training schemes using lower repetitions with heavier loads, lower volume and longer rest periods (Ahtiainen et al., 2005; Goto et al., 2005; Häkkinen et al., 1993; Kraemer et al., 1990). Therefore, the high volume, short rest period REB scheme is effective in inducing stress on the body to a degree that causes a greater reliance on glycolysis and should be more effective in increasing T concentrations than low volume, long rest period REB schemes.

#### Resistance Training and Session Rating of Perceived Exertion

The session rating of perceived exertion (SRPE) represents a tool that was developed to quantify the athlete's perception of the intensity of the entire exercise session (Foster et al., 1996, 2001). The SPRE has been successfully used as a measure of training volume-load both in cross sectional and training studies (Charro et al., 2010; Dey et al., 2004; Foster et al., 2001). Charro et al. (2010) compared the SRPE and post-pre change in T for two different loading schemes. The researchers did not find a significant difference in SPRE or post-pre change in T between the loading schemes. The results lead the authors to conclude that the volume, which was the same between the two

loading schemes, was the important factor and not the loading schemes in determining the SPRE and post-pre change in T concentrations with a bout of resistance exercise. With a precedence of use and validation with volume and training intensity, SRPE can be used as a research tool for quantifying participant perception of the exercise volume.

### Summary

Testosterone is an anabolic hormone associated with the promotion of skeletal muscle hypertrophy by increasing satellite cell number and protein synthesis. When T concentrations are maximized through high volume ( $\geq 3$  sets of  $\geq 10$  repetitions) and moderate intensity (65-80% of 1-RM) resistance exercise, the chance for T to bind to its receptor is greatly improved. Whether the elevation in T is stimulated by an exercise-related increase in catecholamines or through a change in T concentration due to a shift in plasma volume is still not fully understood.

Cortisol is the primary catabolic hormone responsible for proteolysis during recovery from exercise. Cortisol is elevated in response to high volume *or* high intensity work and can be reduced postexercise bout if protein or carbohydrates are ingested. Cortisol may reduce T concentrations and or compete with T for its androgen receptor. Reducing C post REB is an important factor in promoting skeletal muscle hypertrophy.

While much is understood about the response of T and C to the differing modes, intensities, exercise volume and rest periods prescribed in resistance training, there is little research on the effect of exercise order. An optimal exercise order of large muscle mass before small muscle mass may produce significant increases in T but with lower C values. Such a response from T and C would optimize the anabolic milieu and potentially improve the resulting degree of skeletal muscle hypertrophy. If there is a significantly

different response of T and C as a result of exercise order, is that response due to an increase in the production in T and or C or is the different response a result of changes in plasma volume which concentrate or dilute circulating T and C? There has not been a study that has examined the plasma volume corrected T and C concentrations after two different resistance training exercise orders.

Additionally, if exercise order does affect T and C concentrations, do increases in T and or C result in changes in the exercise volume or alter the perception of the volume of a resistance exercise bout? Charro et al. (2010) did observe that the T response, and SRPE were not elevated when exercise volume was the same. If changes in volume do influence the T and C responses to a different exercise order, is the perception of the volume also modified? Or will changes in T and C responses alter the exercise volume without influencing the perception of the volume?

The purpose of this study is to examine if exercise order is an important factor in elevating T or C. If T or C was elevated or depressed did that change affect exercise volume or the perception of the exercise volume?

## CHAPTER 3

### PROCEDURES AND METHODS

This chapter provides a detailed description of the procedures and methodology used to assess the effect of the ordering of upper (UB) and lower body (LB) resistance exercises during a resistance exercise bout on the total testosterone (T) and total cortisol (C) responses, volume, the session rating of perceived exertion (SRPE) and lactate. Sections for this chapter include participants, study design, procedures, assessments and instruments, and statistical analysis.

#### Participants

Nine healthy adult (19-35 yrs) men with at least 1 year of recreational resistance training experience and possessing a maximum bench press to body weight ratio of 1 to 1 and maximum leg press to body weight ratio of 2 to 1 were recruited for the study. Participants did not exceed 25% body fat in order to minimize the peripheral aromatization of T to estradiol that occurs in men with excess body fat (Vettor et al., 1997). Participants with acute or chronic injuries that limited their ability to perform the exercise protocols were excluded from the study. During the course of the study, participants only engaged in regular submaximal aerobic physical activity ( $\leq 75\%$  of  $VO_{2max}$ ) and refrained from individual resistance training protocols.

Participants with a history of pituitary, renal, hepatic, cardiovascular or metabolic disease or those on a low calorie, low fat or ketogenic diet were excluded from the study

(Durand et al., 2003). With the exception of a multivitamin and protein supplement, cessation of other supplement use (i.e., creatine) was required 1 month prior to and during the study. Additionally, participants with a history of anabolic steroid use were instructed to not use anabolic steroids 6 months (Borer, 2003) prior to the study to help ensure concentrations of circulating hormones were strictly from endogenous sources.

All participants were recruited via email, word of mouth, and print advertisements. Participants were required to review and sign a consent form authorized by the University of Utah's Institutional Review Board.

### Study Design

The study used a quasi-experimental, crossover design. Participants performed both exercise orders (UB-LB and LB-UB) and each participant served as his own control. For every participant the UB-LB order was performed during session 2 and the LB-UB order during session 3. The UB-LB and LB-UB exercise orders were not randomized for session 2 and 3 because of evidence (Ahtiainen et al., 2003, 2005) supporting the 96 and 72 hr wash out periods as long enough time periods for sufficient recovery between session 2 and 3. Additionally, participant burden would have increased because of the additional 2 sessions needed to determine if the ordering of session 2 and 3 produced an order effect.

### Procedures

The study was 7 days long with three exercise sessions (Figure 3.1). Participants refrained from resistance training 1 day prior to session 1 to ensure an accurate measure of their 1-repetition maximum (1-RM). Participants were allowed to engage in resistance

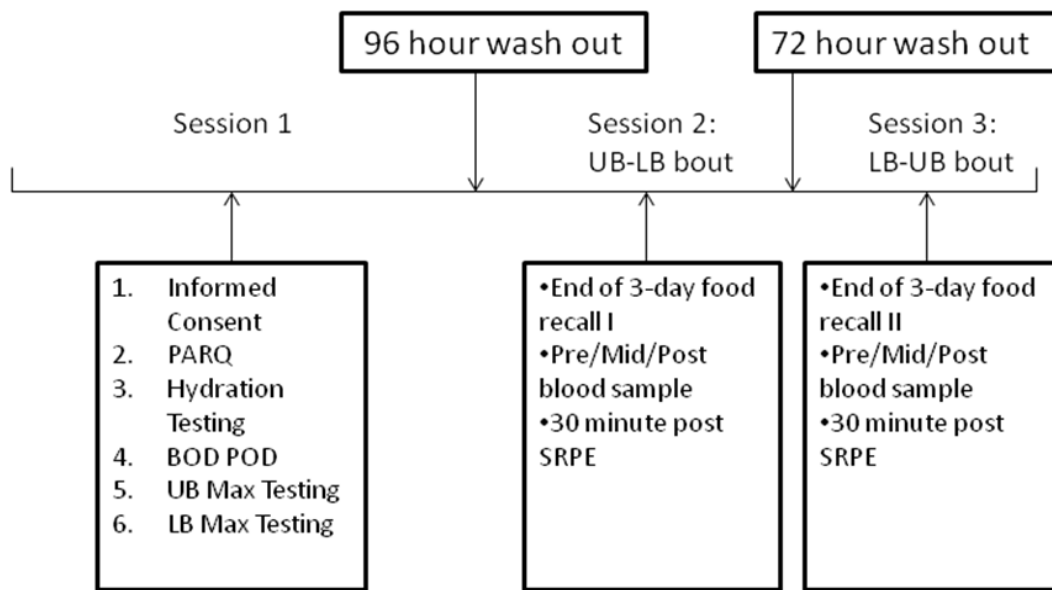
exercise during the remaining hours of the day on which session 1 occurred. A 96-hr no resistance exercise interval was observed between session 1 and session 2 to allow for recovery from the bout of maximum repetition exercise and ensure resting values of T and C prior to session 2 (Kraemer et al., 1998; McCall et al., 1999). The second session consisted of the upper body (UB)-lower body (LB) protocol followed by a 72-hr no resistance exercise washout (McCall et al., 1999). After the 72-hr washout, session 3 was performed and consisted of the LB-UB protocol. The participants were required to wear loose fitting exercise clothing for each of the sessions. Compression shorts, lifting shirts, or other devices that could aid in resistive exercise execution were not permitted.

The importance of hydration was explained to the study participants during session 1. Hydration status was quantified by measuring the specific gravity and pH of the participant's urine (Bayer Multistix 10 SG). The results of the specific gravity and pH test strip were used to determine if the body weight recorded during session 1 was under a euhydrated condition. A state of dehydration during sessions 2 and 3 was defined as a deviation of -2.5% or more from baseline body weight (Judelson et al., 2008).

Participants were allowed to consume water *ad libitum* during all three exercise sessions.

All participants fasted 3 hrs prior to each of the three sessions to minimize effects of acute dietary variables on hormonal concentrations (Brandenberger et al., 1982).

Participants were encouraged to not change their diet and sleep schedules throughout the study. A 3-day food log (Appendix A) was administered at two different times during the study to check for dietary consistency. These 3-day food logs covered the 3 days before both session 2 and session 3 (Figure 3.1). The results of the 3-day food logs were



*Figure 3.1. Timeline for the study.*

compared for total calories and macronutrient densities (SQL Food Processor, ESHA Research, Salem, OR) to control for the influences of diet on endocrine function (Volek et al., 1997).

### Session 1: Familiarization and Maximum Strength Testing

The IRB consent form, supplement and steroid use questionnaire (Appendix B), and Physical Activity Readiness Questionnaire (PARQ) (Appendix C) were administered to the participants in session 1. In addition, height (cm), body weight (kg), age (yrs), and body composition (%fat) (via the BOD POD, Life Measurements Inc., Concord, CA) were obtained for each participant. Body mass distribution equations were used to estimate the proportion of mass located in the UB and LB and as a possible explanation for any differences in T and or C concentrations resulting from either the UB or LB resistance exercise (de Leva, 1996). The current assumption is that the LB, with its greater mass of skeletal muscle, should produce more T and C after a LB resistance

exercise as opposed to the T production after an UB resistance exercise utilizing the smaller muscles of the UB. By using the participant's body weight the difference in the distribution of body mass between the UB and LB was estimated using body mass distribution prediction equations; therefore the influence of the size of the muscle mass on T and C production could be examined (de Leva, 1996).

Session 1 included a general warm-up (5 minutes of low intensity cycling) and four warm up sets leading up to the final set used to determine the predicted maximum. This procedure was repeated for both the UB and LB exercises. The general warm up was done at the beginning of the session followed by the UB familiarization and maximum strength testing and then the LB familiarization and maximum strength testing. For both the UB and LB resistance exercises, the initial loads used during the warm up sets were based upon percentages of the participants self reported estimation of their 1-RM (Table 3.1). The warm up sets leading to the final maximum repetition attempt had a dual purpose of warming up the participant for the maximum repetition attempt and familiarizing the participant with the metronome-established cadence for the repetitions.

Table 3.1

*Sets and Repetitions for Session 1, Familiarization and Maximum Strength Protocol*

Set	Number of repetitions
1: 40-50% of self-reported maximum	10
2: 50-60% of self-reported maximum	10
3: 70% of self-reported maximum	8
4: 80% of self-reported maximum	3
5: $\geq 85\%$ of self-reported maximum	Maximum repetitions but fewer than 3



After the fifth set, additional sets were added if the load for the fifth set allowed for more than three repetitions. The pattern of added sets was continued until a 3-RM was achieved. The goal was to reach a 3-RM set with no more than 3 additional sets. The prediction of 1-RM from a submaximal load is most accurate when the repetitions are low (7-10 repetitions) (Braith et al., 1993). Thus a  $\leq 3$ -RM load for the final set was used as the upper bound because of its assumed stronger relationship to a 1-RM without the challenge and risk of attempting a maximal load (Braith et al., 1993). Only repetitions performed to the cadence of the metronome were counted during the last set. The participant was warned once to keep up with the cadence during the maximum strength testing set. If the participant could not keep up with the metronome cadence, the set was ended and the repetition during which the participant was able to complete on cadence was counted as the final repetition. Most participants reached fatigue quickly and completed all the repetitions they could without slowing from the metronome cadence before reaching a repetition that they could not complete. The completed repetitions during the last set were used to predict the 1-RM for both the UB and LB exercises using the equation from Brzycki (1993):

$$\text{Load (kg)} / 1.0278 - (0.0278 * \text{Repetitions completed}) \quad \text{Equation 3.1}$$

Two to three minutes of rest was observed between sets and 5 mins between exercises. The 1-RM values recorded during maximum strength testing of session 1 were used to identify the 73.5% of 1-RM load for both the LB and UB exercises during sessions 2 and 3. The percentage of 73.5% was selected because of its correspondence to

a 10-RM load (Baechle, 1994). Ten repetitions per set was selected because high volume sets ( $\geq 10$  repetitions per set) promote a significant increase in T (Kraemer et al., 1990, Kraemer & Ratamess, 2005).

### Sessions 2 and 3: UB-LB and LB-UB Exercise Orders

To determine if there was an interactive effect for exercise order on T and C concentrations, participants performed two REBs utilizing a different order of resistance exercise per REB. The first order, a UB-LB order was performed during session 2 and will be known as the UB-LB order. The LB-UB order was performed during session 3 (Figure 3.1) and will be known as the LB-UB order. In between the LB-UB order and the UB-LB order, a 72-hr nonresistance exercise wash out was observed to reduce the potential for carryover of any fatigue or soreness into the LB-UB order from the UB-LB order (Clarkson et al., 1992) (Figure 3.1).

A general cardiovascular warm-up of 5 minutes on a cycle ergometer preceded both REB orders using the same speed and resistance as established in session 1. A set of 10 repetitions using a load at 50% of 1-RM was used before each exercise in the UB-LB REB in session 2 and the LB-UB REB in session 3. The LB and UB resistance exercises consisted of 4 sets of maximal repetitions per set with a training intensity of 73.5% of 1-RM (Baechle, 1994). There was a 1.5-min rest between all sets (Kraemer et al., 1987; 1990; 1991; Kraemer, Kilgore et al., 1992) and a 5-min rest between the LB and UB exercises.

### Resistance Exercises

For both resistance exercises, repetitions were performed continuously with a 1 second concentric and 1 second eccentric phase for each repetition. The timing of the concentric and eccentric phase was established with a metronome set at 60 beats/min, a cadence used in previous studies to reduce the contribution of momentum during the exercise (Crewther et al, 2006; Kraemer, Kilgore et al., 1992). To ensure consistency between sessions, hand placement for the barbell bench press and feet placement for the leg press were measured and recorded. Spotting techniques for each exercise, as described by Baechle (1994), were used to ensure the safety of the participants. The investigator watched each repetition and provided verbal feedback to ensure that for each repetition there was a complete range of motion.

The resistance exercises utilized were the barbell bench press (UB) and the reclined leg press (LB) (FreeMotion Epic, Logan, UT). The full range of motion for the bench press consisted of both the concentric and eccentric actions. The concentric action constituted the full extension of the elbows, as a result of concentric action of the primary movers: pectoral muscles, triceps and anterior deltoid. The lowering of the barbell to the chest around the area of the nipples constituted the end of the eccentric movement phase. During the concentric movement participants were encouraged to press the barbell back toward the rack pins as the bar ascended. During the bench press the head, upper back and buttocks remained in contact with the bench while the feet remained in contact with the floor (Baechle, 1994). The grip width used during the bench press was self-selected by the participant, but was consistent for both sessions 2 and 3.

For the leg press, participants completed the concentric movement phase of the exercise when the knees reached 175° (5° from full extension) extension. The completion of the eccentric portion of the exercise was achieved with 90° of knee flexion or if posterior pelvic tilt occurred (the lower back left the seat back). The investigator watched each repetition and provided verbal feedback to ensure a completion of both the concentric and eccentric phases of the leg press. The position of the feet was self-selected by the participant, but was consistent for both sessions 2 and 3. Only successful repetitions, as previously outlined for each exercise, were counted.

### Volume

In order to assess the effect of changes in T on total work during sessions 2 and 3, a computation of work done during sessions 2 and 3 can be made. However, in a practical weight training setting, total work is not generally measured whereas surrogates for work, intensity (% of 1-RM) and volume (sets x repetitions per set), are measured (Baechle, 1994; Bompa et al., 2003). As mentioned intensity (50% of 1-RM for the warm up, 73.5% of 1-RM for the protocol) was constant during the warm up and REBs while volume was allowed to vary if there was fatigue from performing 4 sets of a 10RM load per exercise. Repetitions to failure has been shown to produce a significant increase in T, thus each set was performed to failure (Häkkinen et al., 1993). If a significant difference in circulating T was found with the LB-UB or UB-LB orders along with a significant difference in volume, then a conclusion could be drawn that the ordering of UB and LB resistance training exercises effects acute changes in T that may affect volume.

### Blood Sampling

To limit effects of diurnal variation on both T and C concentrations, sessions 1, 2, and 3 took place at the same time of day (Borer, 2003) for each participant (Häkkinen et al., 1993; Thuma et al., 1995; Veldhuis et al., 1987). The submaximal cardiovascular warm up was performed before any blood draws to separate the influence of the resistance exercises performed from the influence of the cardiovascular warm up on the measurements of T, C, lactate, hemoglobin and hematocrit. A 2-min rest period was observed after the submaximal cardiovascular warm up before the first blood draw was taken. All blood draws were taken via venipuncture by an individual trained and experienced in doing venipunctures.

Blood samples were taken from an antecubital vein using a 21-gauge needle and vacutainer set up (BD Vacutainer® SST<sup>TM</sup> Tiger Stopper) at pre-, mid- and immediately postsession 2 and 3 (Figure 3.1). Samples were spun at 600-800g within 35-65 minutes (Chance et al., 2009) of gathering the sample. Serum was aliquoted out into cryovials and stored within 80 minutes of the pre-session draw. All samples were stored at -80°C until assayed.

### Assessments and Instruments

Serum samples were assessed for T and C concentrations by ARUP Diagnostic Laboratories (Salt Lake City, UT) utilizing the electrochemiluminescent immunoassay method for T and the chemiluminescent immunoassay method for C. The sensitivity of the T analyses was reported as 0.04ng/mL with an interassay coefficient of variance (CV) of 4.6-5.6% (Personal communication, ARUP Diagnostic Laboratories). The sensitivity of the C analyses was reported as 0.02µg/dl with an interassay CV of 5.9-6.4% (Personal

communication, ARUP Diagnostic Laboratories).

A small blood sample captured in a microcapillary tube was taken via finger prick at the same time as every venipuncture. The microcapillary tube sample was measured for total blood quantity and for cell quantity in order to obtain a hematocrit value for each pre-, mid- and postsession sample.

A hemoglobin analyzer (Hemocue 201b, Sweden) was used to determine hemoglobin concentration at every time point. The hematocrit and hemoglobin values for each time point were used in the Dill and Costill (1974) equation to estimate the acute exercise-related changes in plasma volume resulting from sessions 2 and 3. Testosterone and C were examined as both uncorrected and corrected for the exercise-related changes in plasma volume to determine whether any of the potential exercise-related concentration of T and/or C may be explained by the acute exercise-related changes in plasma volume (Kraemer, Kilgore et al., 1992).

#### Session Rating of Perceived Exertion

A session rating of perceived exertion (SRPE) (Foster et al., 1996; Dey et al., 2004) was used to quantify the perceived effort of the UB-LB or LB-UB orders. The measure of SRPE was recorded 30 min after completion of the UB-LB or LB-UB orders to ensure the perceptual rating was reflective of the whole session and not just the effort perceived due to the last set (Dey et al., 2004). The scale used for the SRPE (Appendix D) has been shown to be a valid and reliable estimate of session exercise intensity for both endurance and resistance exercise sessions (Dey et al., 2004; Foster et al., 1996).

### Statistical Analysis and Sample Size Estimation

A mixed-factor repeated measures analysis of variance (RMANOVA) model was used to assess the interactive effects of the UB-LB vs. LB-UB orders for the uncorrected and the plasma volume-corrected T and C concentrations. For any significant two-way order by time interactions observed between plasma volume corrected and uncorrected concentrations of T and C that required further interpretation, paired *t*-tests with an alpha level of 0.05 were used to determine specific mean differences. Using the results from Volek et al. (1997), the effect size for the difference in the T response to upper and lower body resistance exercise was estimated as 0.80. Experimental alpha was set to 0.10, which was Bonferroni-corrected to  $p = 0.025$  for each of the 4 study outcomes. Six participants were required to detect the hypothesized effect size with >80% power (GPower; version 3.0; Kiel, Germany). The experimental alpha was set to 0.10 because of the highly conservative nature of the Bonferroni correction (Bland & Altman, 1995).

A paired *t*-test was used to assess the effect of exercise order, UB-LB vs. LB-UB, on the total volume performed for both exercise orders. Additional paired *t*-tests were used to assess the effect of exercise order, UB-LB vs. LB-UB, on the perception of effort, measured with SRPE, for both exercise orders. The order by time interaction for lactate concentrations was also analyzed using a RMANOVA. Experimental alpha was set to 0.05 for both paired *t*-tests. All statistical analyses were done using SPSS (version 17.0) statistical software (Chicago, IL) and Microsoft Excel (2007 version) (Seattle, WA).

## CHAPTER 4

### RESULTS AND DISCUSSION

This chapter presents the results from the procedures and data analyses outlined in Chapter 3. This chapter includes participant characteristics, the order by time interaction of both plasma volume corrected and uncorrected testosterone (T) and cortisol (C) responses to the UB-LB and LB-UB orders, the comparison of total exercise volume between the UB-LB and LB-UB orders, the comparison of the session rating of perceived exertion recorded after each exercise order and the order by time interaction of lactate concentrations during both exercise orders.

#### Results

##### Participant Characteristics

The demographic data for the sample are presented in Table 4.1. The average age of the sample of 9 adult men was  $27 \pm 4$  yrs. All the participants indicated they had participated in at least 1 year of resistance training. None of the 9 participants reported using steroids 6 months prior to the study and they all agreed to refrain from using supplements other than protein powder and multivitamins. All the participants met the required strength to body weight ratio for the bench press of 1:1 and the strength to body weight ratio for the leg press of 1:2 (Table 4.1). Normal T concentrations were observed for all 9 participants with no participant below the lower value of 320ng/dl (Buresh et al.,



Table 4.1  
*Descriptive Data for the Sample of 9 Adult Men*

Variable	$M \pm SD$
Age (yrs)	27 $\pm$ 4
Height (cm)	174.9 $\pm$ 7.8
Body Fat %	14.3 $\pm$ 5.7
Baseline Hydration	
Specific Gravity	1.01 $\pm$ 0.01
pH	5.9 $\pm$ 0.6
Strength Values	
Bench Press Maximum (kgs)	97.72 $\pm$ 15.91
Relative Bench Press Maximum (bench maximum/body mass)	1.26 $\pm$ 0.14
Leg Press Maximum (kgs)	294.09 $\pm$ 65.46
Leg Press Maximum (leg press maximum/body mass)	3.89 $\pm$ 0.73

2009; Fahey et al., 1976; Rosner et al., 2007). The mean pre-session T value was 559 $\pm$ 132ng/dl, a value similar to T values reported elsewhere for young males (Bhasin et al., 2001).

Prior to the study, participants were determined to be euhydrated by using urine dip strips and a specific gravity index of 1.01 $\pm$ 0.1 and pH of 5.9 $\pm$ 0.6 that according to the manufacture of the urine dip strips (HydraTend, UriDynamics, Inc., U.S.A.) indicates euhydration. Participants were not dehydrated ( $\geq$ 2.5% decrease in body weight) before the UB-LB order or the LB-UB order based upon the standard of  $\geq$ 2.5% decrease in body weight (Table 4.1). The amount of sleep obtained on the night before a session was not different between sessions 1, 2 and 3. Body composition values met the inclusion criteria for the study ( $\leq$ 25% body fat).

The starting time of sessions 2 and 3 were not significantly different (Table 4.2). Additionally, the total duration of both sessions 2 and 3 was the same (Table 4.2). Dietary patterns for the participants between sessions 1 and 2 and session 2 and 3 were not significantly different (Table 4.2).

#### Testosterone and Cortisol Responses to the Upper Body-Lower Body and Lower Body-Upper Body Orders

Because ARUP misplaced one of the pre time points for one of the participants, a mean replacement technique was used for both the T and C values lost with the missing sample. The T responses to the UB-LB or LB-UB orders are shown in Figure 4.1. Mauchly's test for sphericity was not significant for the order by time interaction ( $p = 0.13$ ). There was not a significant difference for order by time interaction ( $p = 0.08$ ).

The C responses to both the UB-LB and LB-UB orders are shown in Figure 4.2. Mauchly's test for sphericity was not significant for the order by time interaction ( $p = 0.32$ ). A significant difference was not seen for the order by time interaction ( $p = 0.17$ ).

Post hoc power analysis revealed that the order by time interaction for T (0.31 and .50, respectively) and for C (0.07 and 0.36, respectively) was not statistically powered.

#### Plasma Volume Corrected Testosterone and Cortisol Responses to the Upper Body-Lower Body and Lower Body-Upper Body Resistance Training Bouts

Plasma volume changes as computed from the equations from Dill and Costill (1974) were completed using a measure of hemoglobin and the hematocrit. The percent changes in plasma volume are shown in Table 4.3. Post hoc power analysis revealed that

Table 4.2

*Bodyweight, Hours Slept, Session Start Time and  
Dietary Values Over the Course of the Study*

Variable	$M \pm SD$
Weight (kg)	
Session 1	77.63±9.95
Session 2	78.68±9.85*
Session 3	78.91±9.49*
Hours Slept (hrs)	
Session 1	7.6±1.0
Session 2	7.4±0.9
Session 3	7.3±0.7
Session Start Time (hrs:mins)	
Session 2	10:33:00±2:04
Session 3	10:30:00±2:07
Total Session Time (mins)	
Session 2	23±2
Session 3	24±2
3-Day Food Recall	
Total Calories (kcal)	
Session 1	9293±3449
Session 2	8494±2001
Calories from Carbohydrates (%)	
Session 1	51.1±5.2
Session 2	54.5±6.5
Calories from Protein (%)	
Session 1	16.0±2.6
Session 2	17.4±4.4
Calories from Fat (%)	
Session 1	32.7±5.9
Session 2	28.1±7.0

\*Significantly different ( $p < 0.05$ ) from session 1 value.

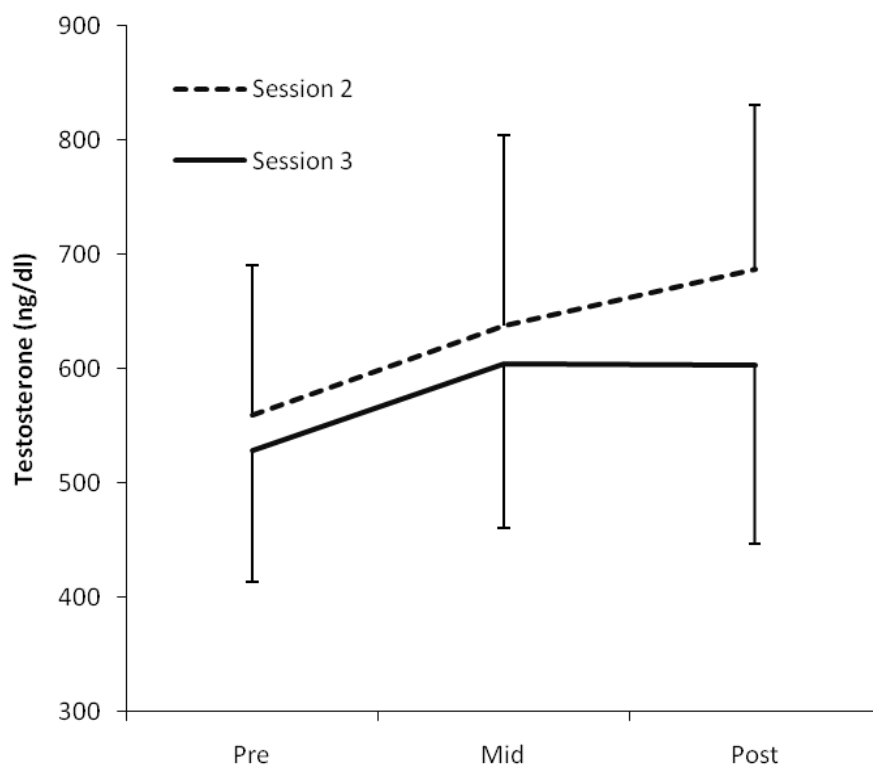


Figure 4.1 Testosterone concentrations at pre, mid and post both the UB-LB and LB-UB exercise orders.

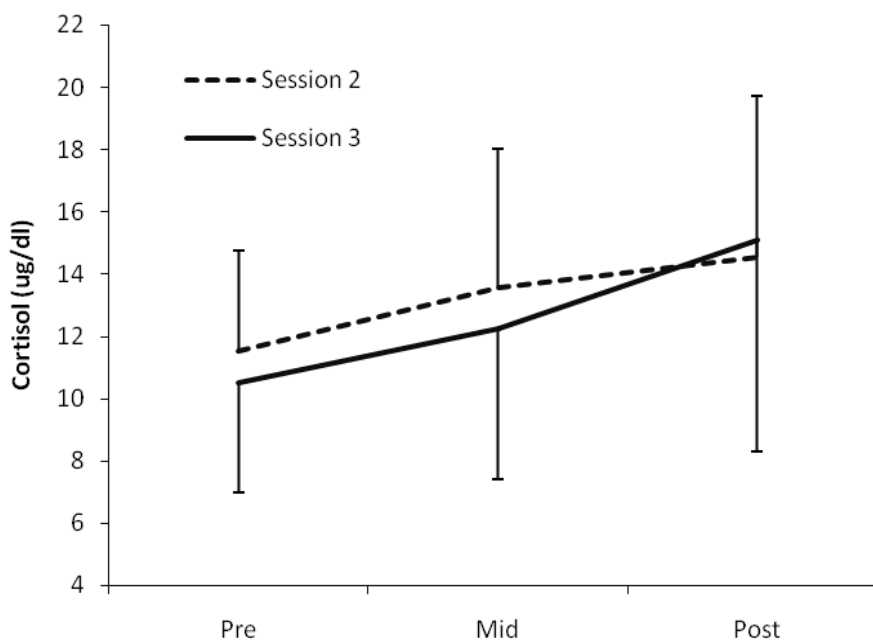


Figure 4.2. Cortisol concentrations pre, mid and post both the UB-LB and LB-UB exercise orders.

Table 4.3

*Hemoglobin (Hb), Hematocrit (Crit) and Changes in Plasma Volume (PV%Δ) for Both the UB-LB and LB-UB Exercise Orders*

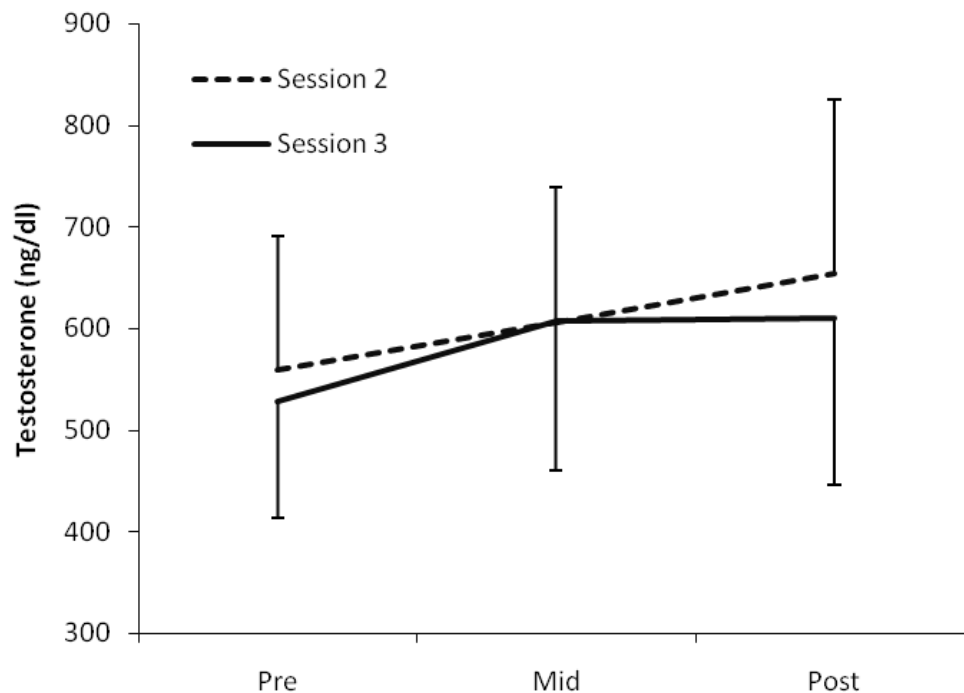
Variable	Hb (gm/dl)	Crit (%)	PV %Δ
UB-LB			
Pre	16.5±1.0	51.3±2.7	-
Mid	17.2±1.1	50.8±2.2	(4.2)±9.3
Post	18.1±1.2	50.8±2.0	(5.4)±9.5
LB-UB			
Pre	16.7±1.2	52.6±1.6	-
Mid	17.3±1.2	50.6±2.3	0.7±5.6
Post	17.6±1.2	48.4±4.2	1.2±14.2

the order by time interaction for T (0.31 and .50, respectively) and for C (0.07 and 0.36, respectively) was not statistically powered.

Plasma Volume Corrected Testosterone and Cortisol Responses to the Upper Body-Lower Body and Lower Body-Upper Body Resistance Training Bouts

Plasma volume changes as computed from the equations from Dill and Costill (1974) were completed using a measure of hemoglobin and the hematocrit. The percent changes in plasma volume are shown in Table 4.3.

Plasma volume corrected T responses to both the UB-LB and LB-UB orders are shown in Figure 4.3. Mauchly's test for sphericity was not significant for the order by time interaction ( $p = 0.32$ ). A significant difference in the order by time interaction was not observed ( $p = 0.62$ ).



*Figure 4.3. Plasma volume corrected testosterone concentrations pre, mid and post UB-LB and LB-UB exercise orders.*

Plasma volume corrected C responses to both the UB-LB and LB-UB orders are shown in Figure 4.4. Mauchly's test for sphericity was significant for the order by time interaction ( $p < 0.01$ ). There was not a significant difference for the order by time interaction ( $p = 0.22$ ).

Post hoc power analysis revealed that the order by time interaction for plasma volume corrected T (0.12 and 0.16, respectively) and C (0.05 and 0.23, respectively) were not statistically powered.

#### Volume and SRPE and the Upper Body-Lower Body and Lower Body-Upper Body Resistance Training Bouts

There was a nonsignificant difference in total exercise volume between the UB-LB and LB-UB exercise orders (Table 4.4). The perception of the exercise volume for the

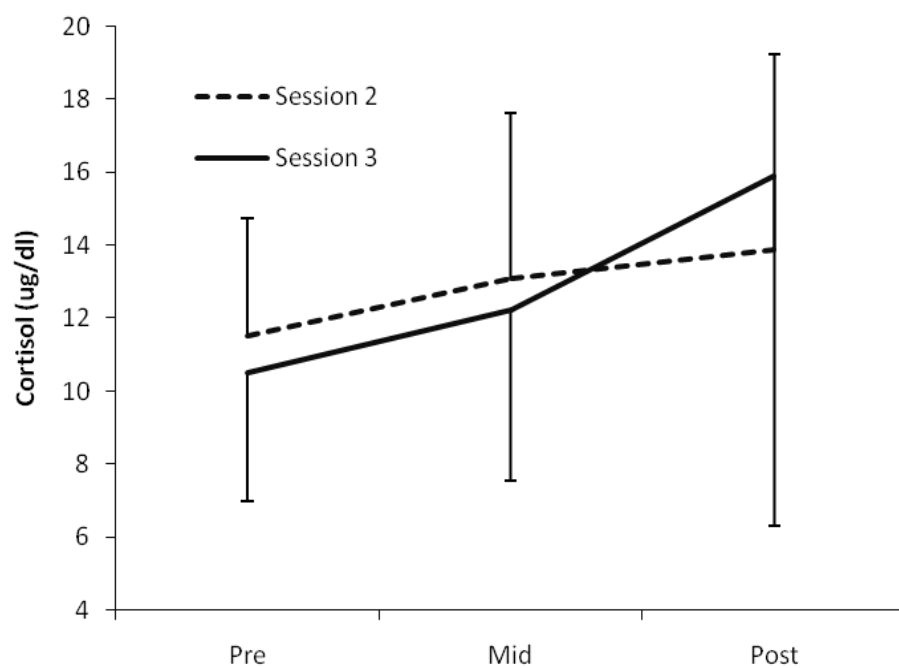


Figure 4.4. Plasma volume corrected cortisol concentrations pre, mid and post both UB-LB and LB-UB exercise orders.

Table 4.4

*The Total Exercise Volumes for the UB-LB and LB-UB Orders Were not Significantly Different*

Variables		Repetitions
UB-LB		
Bench Press		29±4
Leg Press		33±6
	Total	
Volume		62±7
LB-UB		
Leg Press		36±8
Bench Press		25±3
	Total	
Volume		61±7

Note.  $p = 0.61$ .

UB-LB and LB-LB exercise orders were quantified using SRPE (Figure 4.5). There was not a significant difference between the mean SRPE value between the UB-LB order ( $7.2 \pm 0.9$ ) and the LB-LB order ( $6.7 \pm 1.4$ ) (Figure 4.5). Blood lactate was also used to quantify the intensity of the REBs (Figure 4.6). The order by time interaction for lactate was not significant ( $p = 0.14$ ).

### Discussion

The primary objective of this study was to determine if exercise order influenced either plasma volume uncorrected and corrected concentrations of T and C. The hypothesis was that there would be a significant order by time interaction for plasma volume uncorrected T values, but the resultant data did not support this hypothesis. Testosterone was elevated by 18% for the UB-LB order and by 12% for the LB-LB order, T increases that are similar to the 16% increase reported in a study using a combination of upper and lower body resistance exercises (Spiering et al., 2009). Furthermore, the magnitude of the increase in T as a result of the high volume, repetitions to failure and short rest period scheme characteristic of the current study is in agreement with other studies utilizing a similar resistance training bout scheme (Ahtianen, Pakarinen, Kraemer et al., 2003; Häkkinen et al., 1993; Kraemer et al., 1990; Smilios et al., 2003).

Although the strategy of using high volume, repetitions to failure and short rest periods successfully increased T, the ordering of the UB and LB exercises did not alter the REB T responses (order by time interaction  $p = 0.08$ ). However, the partial eta squared value for the variance of the delta T scores was  $\eta^2 = 0.30$ , a value indicating that exercise order was producing a modest effect on the T response.



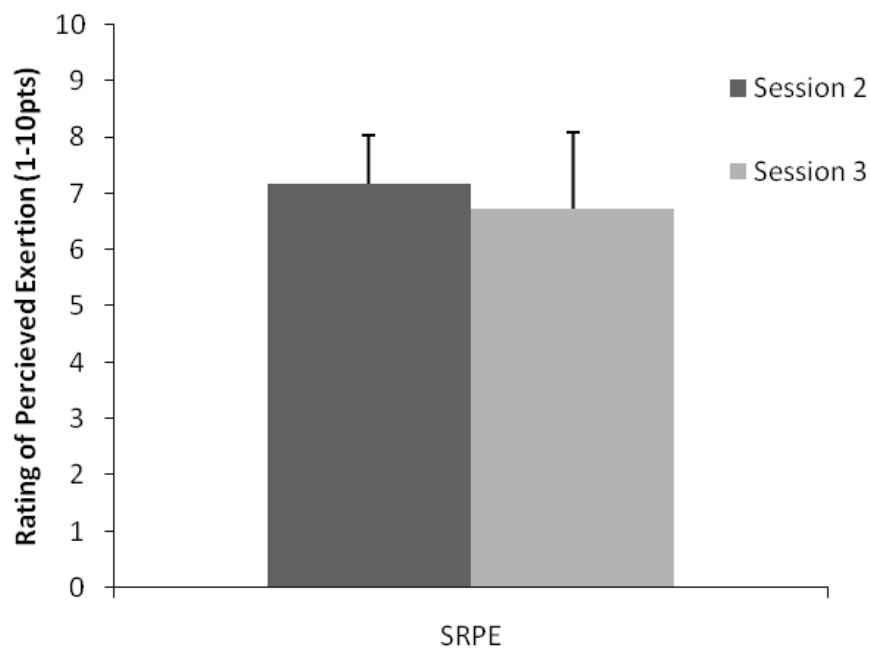


Figure 4.5. Session rating of perceived exertion for the UB-LB order and the LB-UB order. A score of 10 is classified as “maximal” and 1 is classified as “very, very easy.” Values were not significantly different between sessions 2 and 3.

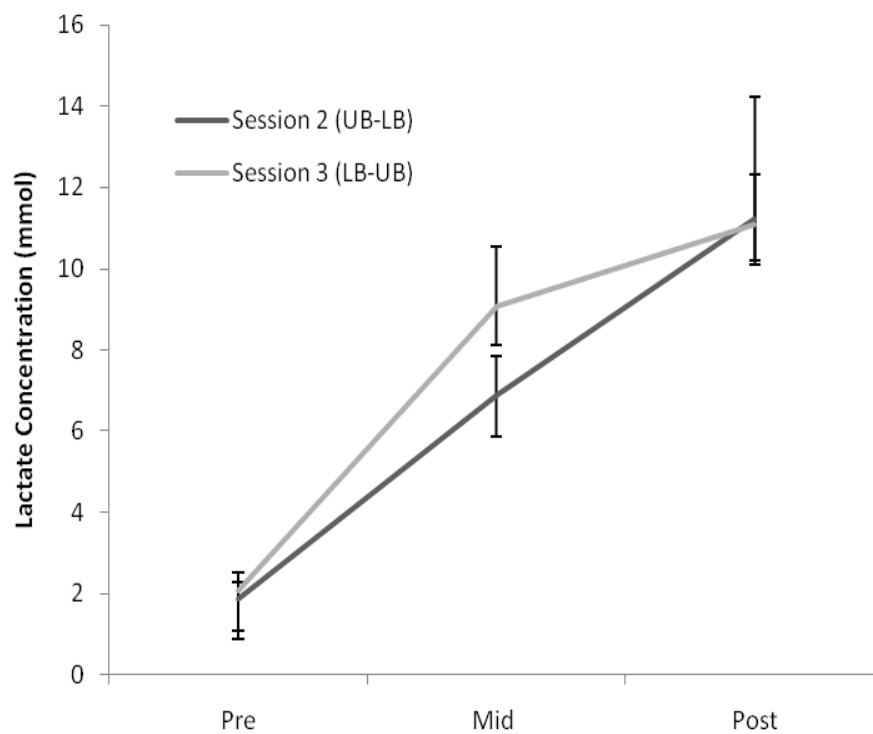


Figure 4.6. Lactate concentrations pre-, mid- and postsession for both the UB-LB exercise order and the LB-UB exercise order.

The nonsignificant order by time interaction ( $p=0.08$ ) may be a reflection of the intraparticipant variability in T response to exercise order. In Figure 4.7, the mean delta T for the mid- to pre-session comparison for the LB-UB order is larger than the mean delta T for the mid- to pre-session comparison for the UB-LB order, but the large 95% confidence intervals explain the lack of statistical significance.

When assessing hormone values in response to exercise, Beaven et al. (2008) has suggested that there will be participants who are responders and nonresponders. The large 95% confidence intervals in Figure 4.7 supports the interpretation that some of the participants were responders, experiencing a very large increase in T in response to the LB-UB order while other participants were nonresponders, not experiencing the same magnitude of response to the LB-UB order. Figure 4.8 presents the concept of responders and nonresponders in a different way. From Figure 4.8 it is clear that all the participants except 1 had a greater T response to the UB-LB order than the LB-UB order.

However, the range of each participant's T response to the UB-LB order was variable with some participants producing little to no T response (nonresponders), while other participants produced responses  $>100\text{ng/dl}$  (responders). The variability of the T response is washed out when low T response values are combined with high T response values producing a mean T response that does not represent either the responder or non-responder.

Another factor that might have contributed to the lack of an exercise order effect is that the T responses to the UB-LB and LB-UB exercise orders may have been influenced by the exercise volume performed during UB-LB and LB-UB orders. To further examine the potential relationship between the exercise volume and the individual

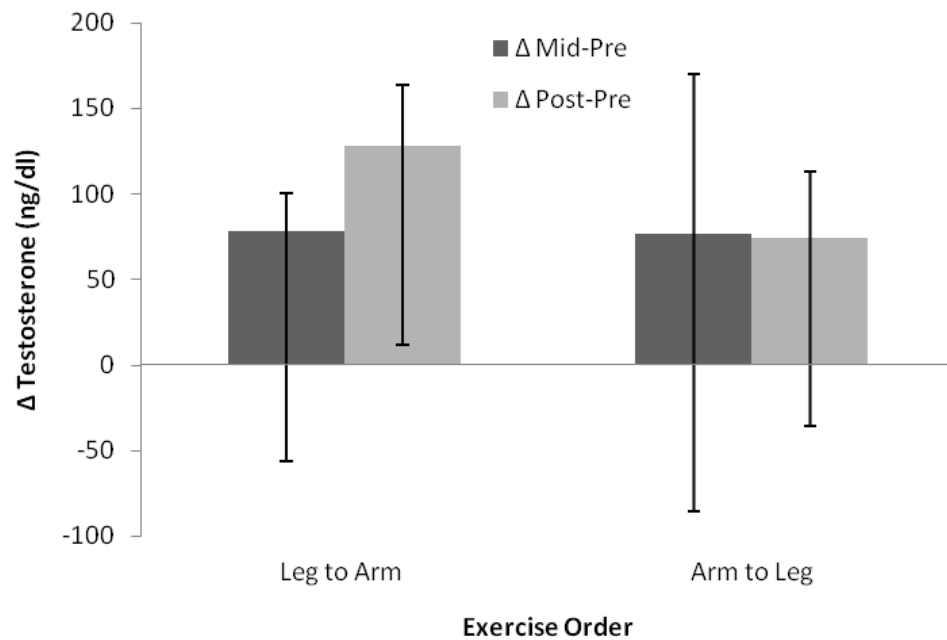


Figure 4.7. Intraindividual difference in  $T$  pre- to midsession and pre- to postsession for both the UB-LB exercise order and the LB-UB exercise order. A 95% Confidence Interval is represented by the bars around each mean.

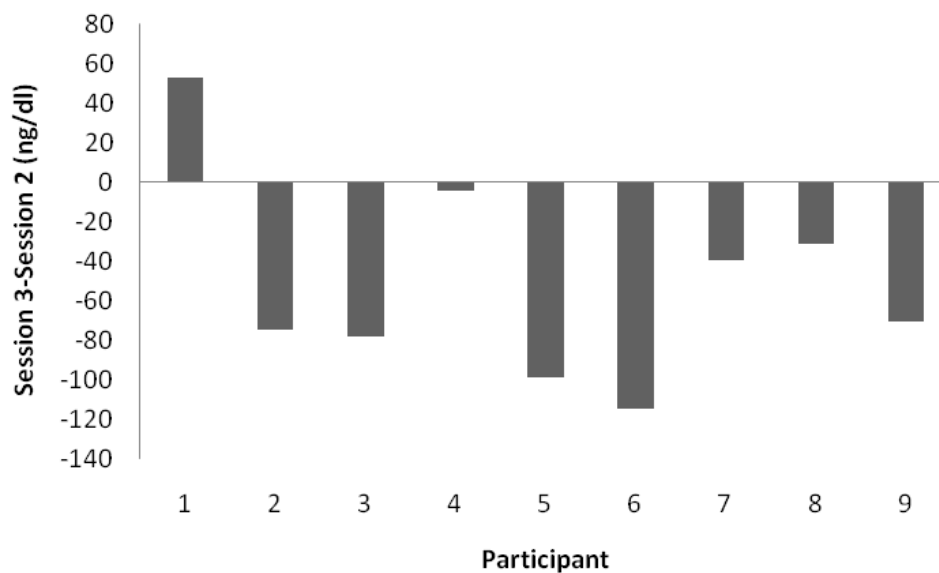


Figure 4.8. Individual difference in  $T$  between the UB-LB exercise order and the LB-UB exercise order (3-2). Individual change in  $T$  is post-pre session plasma volume uncorrected  $T$ . A positive value indicates the UB-LB order produced a greater  $T$  response. The reported  $T$  values had the 5.1% average interassay error removed for each value.

T response for the two exercise orders used in the study, a Pearson's  $r$  correlation was performed. The analyses revealed a moderate ( $r = 0.50$ ) relationship between the individual T response and the individual total volume from the UB-LB and LB-UB orders (Figure 4.9). The relationship in Figure 4.9 indicates that the greater the total volume performed during the UB-LB and LB-UB order, the greater the T response from the UB-LB and LB-UB order pre- to postsession, respectively. For example, as an individual performed more repetitions using the UB-LB order the T response increased as a result of the UB-LB order.

Individual baseline T values might also be a variable that affects the T response, independent of exercise order. Those participants with lower baseline T values did not have observed changes in T regardless of the exercise order. Participants with higher baseline T values did see variation between pre and mid or mid and post time points for both the UB-LB and LB-UB orders (Figure 4.10). The possible effect from the baseline T values on the T response to exercise, again emphasizes the importance of individual response variability.

In a study that did account for individuality in T responses, Beaven et al. (2008) demonstrated that if the combination of volume and intensity for resistance training is optimized for each participant, T responses to exercise are significantly increased along with improvements in strength and muscle mass. Therefore, because all participants performed the same two orders of resistance exercises in the current study, the results do not allow for optimal individual T response leading to the large standard deviation values for each time point and the lack of statistical significance for the order by time interaction. Other studies in the literature have noted that individual variability is a

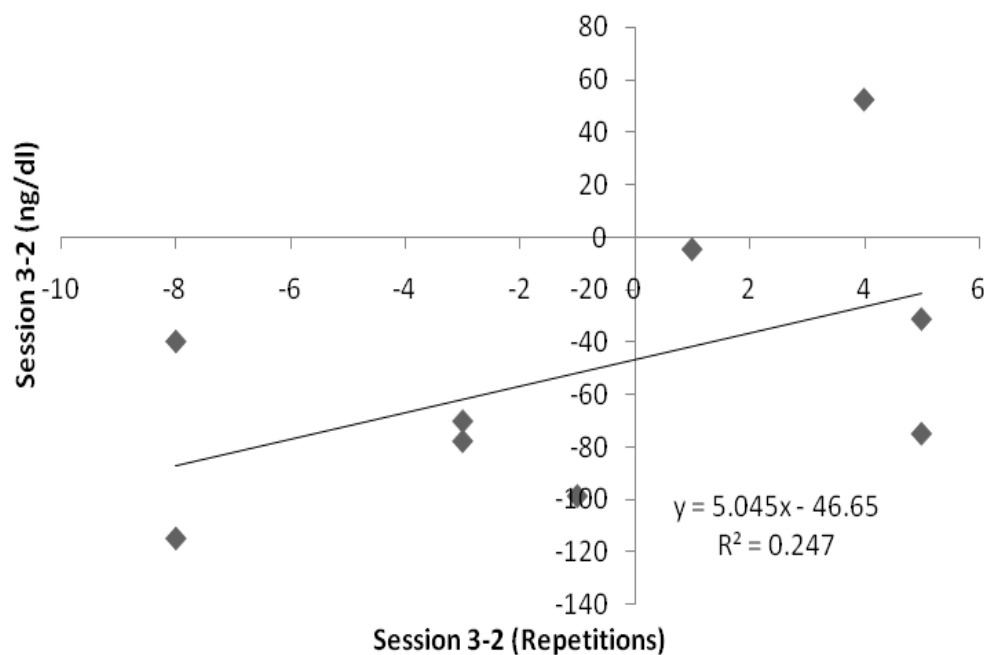


Figure 4.9. Correlation between the individual differences in plasma volume uncorrected T between sessions 2 and 3 and the individual difference in total volume from sessions 2 and 3.

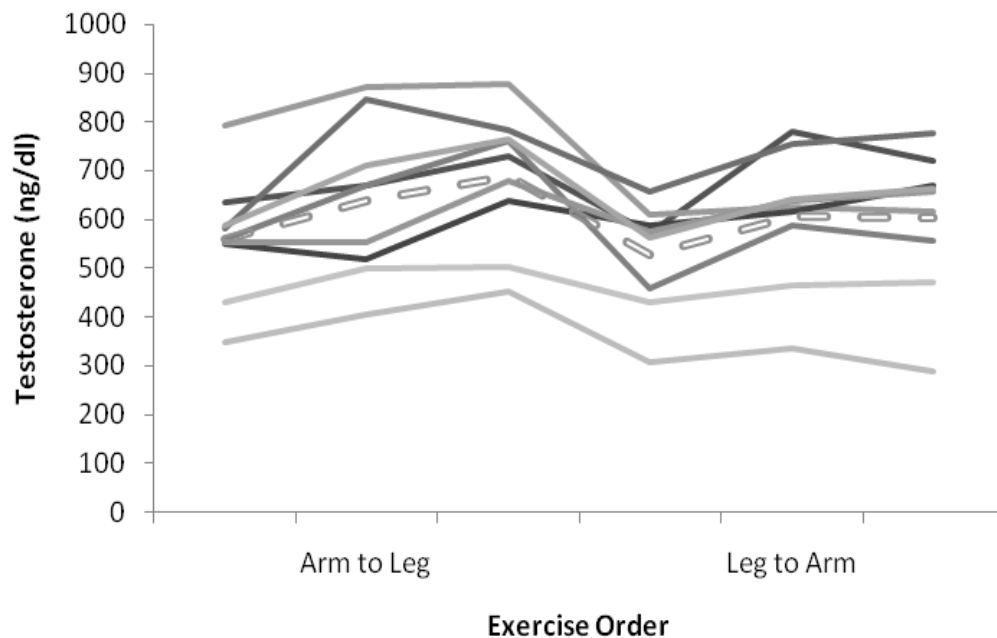


Figure 4.10. T concentrations for each individual at every time point. The dotted line represents the group mean.

potential factor limiting the interpretation of study results. Hansen et al. (2001); Kraemer et al. (1990) and West, Burd, Staples et al. (2010) have all reported large standard deviation values for hormone response time intervals and the clustering of data points in correlation analyses. The large standard deviation values seen in this study reduce the power of the study to detect time by exercise orders interaction. Variability in individual responses in T to a resistance exercise bout (Beaven et al., 2008) may also explain the recent challenges made to the importance of T for increases in skeletal muscle strength and hypertrophy (West, Burd, Staples et al., 2010).

### Secondary Findings

Lactate concentrations were measured in order to determine exercise intensity between the UB-LB and LB-UB orders. The order by time interaction was not significant between session 2 and 3. Together, total volume and lactate are indicators of the volume and intensity of the work performed during a resistance exercise bout (Dey et al., 2004; Kraemer et al., 1990). The nonsignificant interaction for sessions 2 and 3 for lactate and the nonsignificant difference in total volume indicates the stress on the body was similar for both sessions. Moreover, a lack of difference between the session rating of perceived exertion values taken after both the UB-LB and LB-UB orders indicates that participants did not perceive the differently ordered exercise sessions to be different. With no difference in the total volume and perception of the training load between the UB-LB and LB-UB sessions, it is not surprising that the T and C response were not different.

In this study it is possible that during the LB-UB order any improvement in bench press volume or elevation in T by performing the leg press first was negated by the fatigue accrued from the leg press. During the UB-LB order, exercising the smaller

muscles of the upper body during the bench press may not have fatigued the lower body muscles used during the subsequent leg press. However, despite the differences in the distribution in the repetitions between the bench press and leg press the total exercise volume was the same for the UB-LB and LB-UB orders. Therefore, the ordering of the resistance exercises, while possibly influencing the exercise volume of the individual exercises, does not influence total exercise volume.

Although the individual exercise volumes were different between the UB-LB and LB-UB orders, the difference did not result in an order by time interaction of T or C. It is likely that the exercises selected for this study did not utilize a total muscle mass that was different (large vs. small) enough to elicit a significant difference in the T response. Therefore, there is no advantage when separately performing either the leg press or the bench press in attempting to produce a greater circulating T concentration. Previous studies have reported a significant increase in T when comparing an upper body only resistance exercise bout with an upper body plus lower body resistance exercise bout (Hansen et al., 2001; Spiering et al., 2009; West, Burd, Tang et al., 2010). In two of the studies that added a lower body resistance exercise, a leg press was added to the small muscle, single joint bicep curl which served as the upper body exercise (Hansen et al., 2001; West, Burd, Tang et al., 2010). While the addition of the two exercises was done to answer a different question about the importance of T for muscle hypertrophy, the importance of adding a large muscle, multiple joint exercise such as the leg press to the small muscle, single joint exercise biceps curl was assumed to be a catalyst for a greater T response. Based on the results of the studies by Hansen et al. and West, Burd, Tang et al. that hypothesis appears to be true.

However, when the upper body exercise is a multiple joint and larger muscle mass exercise, as with the bench press exercise in this study, the difference in the T response between the two body segments disappeared. The difference between the response of T from the upper and lower body exercises was also assessed relative to the mass of the musculature involved in the bench press and leg press. The upper body mass was estimated as 21.5% of total body mass (de Leva, 1996). The average change in the T concentration was 5.3ng/dl per kg of the upper body mass when using the T response from the bench press performed first in the UB-LB order. Conversely, the lower body muscle mass was estimated as 31% of total body mass (de Leva, 1996) and produced an average change in T of 3.3ng/dl per kg of the lower body mass when using the concentration in T after the leg press performed first in the LB-UB order. These relative changes in T concentrations per kg of either upper or lower body mass were not significantly different ( $p = 0.37$ ). The results of the previous studies coupled with this study support the lay press's assumption (Johnson, 2007) that it is the total amount of muscle mass that is important in elevating the T response. The difference in muscle mass exercised during the bench press and leg press was not great enough to elicit a significant difference in the T response between the two exercises.

The results of this study also did not answer the question about the importance of the sympathetic nervous system and T production. However, this study may support the role of lactate on increasing T concentrations. The nonsignificant order by time interaction between the UB-LB and LB-UB orders combined with the non-significant order by time interaction for T supports a relationship between lactate and the T response to exercise (Lin et al. 2001; Lu et al., 1997).



Taken together the evidence from this study supports the premise that the size of the working skeletal muscle mass is an important contributor to the T response to resistance exercise while lactate may not be a mechanism for T production. Because a significant order by time interaction was not found between the UB-LB and LB-UB order, the importance of resistance exercise order in elevating T is in doubt.

#### Cortisol Response to Exercise Order

The C response to both the UB-LB and LB-UB orders revealed that if a reduction in C was desired to reduce the catabolic environment, it did not matter if the bench press or leg press was performed first. Although there was a difference in the response of C for the two exercise orders, the order by time interaction was not significant. As with T, a lack of a difference in the size of the muscle mass used during the bench press versus the leg press may have contributed to a nonsignificant order by time interaction in C. However, Hansen et al. (2001) also showed a nonsignificant C response between a bout of biceps curls and leg pressing and a bout of biceps curls alone in resistance trained males. The C response may not be dependent on muscle mass but instead on other factors such as exercise intensity and or duration (Brandenberger & Follenius, 1975).

#### Plasma Volume Corrected T and C

The nonsignificant order by time interaction between plasma volume corrected T and C for the UB-LB and LB-UB orders supports the conclusion that resistance exercise order does not affect either T or C. However, the small decrease during the UB-LB order and slight increase in plasma volume during the LB-UB order (-5.3% to 1%) is in conflict with previous research that shows decreases in plasma volume up to 17% with resistance

exercise (Kraemer et al., 1993; McCall et al., 1999). In a study by Kraemer and colleagues (1993), while plasma volume dropped quickly during the first two upper body resistance exercises of their study, plasma volume started to rise during the last two lower body exercises. The discrepancy between previous studies (Kraemer et al., 1993; McCall et al., 1999) and the current study could be due to a lower volume used during the current study (8 sets to failure) as compared to previous research (14 sets to failure) (Kraemer et al., 1993). However, the study by Kraemer et al. (1993) did observe a rise in plasma volume toward the end of the four exercises performed during the study with lower body exercises performed last. All four exercises utilized 3 sets to repetition failure with a 10-repetition maximum load. The rise in plasma volume during the lower body exercises in the study by Kraemer and colleagues may have been as a result of a rise in circulating metabolites as a result of large muscle mass exercise. The metabolites might have attracted plasma out of the intravascular space to the extravascular space.

A similar trend was observed in this study when the bench press was performed first during the UB-LB order and plasma volume dropped 4%. During the LB-UB order, when the leg press was performed first, plasma volume remained unchanged. The difference between the plasma volume response between the bench press and leg press may have been further exacerbated by the significantly greater exercise volume performed during the leg press in the LB-UB order than the bench press in the UB-LB order.

Body position during the bench press and leg press may also have been a contributing factor to the lack of change in plasma volume (Kraemer et al., 1993). Because both exercises were performed in a supine or reclined position and rest was

either in a seated or reclined position, the arterial pressure and thus capillary hydrostatic pressure may have been lower than if other multiple joint exercises performed in an upright posture had been used. If lower hydrostatic pressure was a factor, the lower pressure could have resulted in a reduction of plasma leaving the extravascular space (Kraemer et al., 1993).

Another contributing factor to the slight increase in plasma volume could be as a result of a greater water intake during the LB-UB order. However, with REBs only lasting 20 mins and no more than 20 fluid ounces of water being ingested during the REB, it is unlikely that hydration status made a significant contribution to plasma volume.

#### Limitation of Statistical Power

A factor in the nonsignificant order by time interaction in T and C concentrations observed as a result of the two exercise orders was a lack of statistical power. However, even if the study had been powered ( $>0.80$ ) the partial eta of the T response to the UB-LB and LB-UB orders was only modest for the order by time interaction. With only a modest partial eta value, the meaningfulness of any change in T might be questioned for its practical significance, even if the results had been statistically significant. The other order by time interactions examined in the study for both plasma volume corrected and uncorrected C concentrations and plasma volume corrected T were also underpowered. Unlike the plasma volume uncorrected T response these three order by time interactions produced only weak partial eta squared values. Even if the order by time interactions were powered, the resulting effect size would probably have been trivial.

### Exercise Volume and Session Rating of Perceived Exertion

The secondary objectives of this study were to determine if an elevation in T and C values affect the training volume or the perception of the training volume as measured by session rating of perceived exertion. The difference in total volume was not significant between the UB-LB and LB-UB orders even though the bench press volume was greater during the UB-LB order. The hypothesis regarding volume was that the LB-UB exercise order would produce a greater volume compared to the UB-LB order. The hypothesis was based upon the idea that the lower body would produce a greater T concentration which the upper body musculature would benefit from when the bench press was done second in the exercise order. Clearly the lower body-upper body order was not effective in bringing about a greater bench press volume.

Like total volume, the UB-LB and LB-UB orders resulted in a nonsignificant difference for SRPE. Even with a drop in the volume for the bench press in the LB-UB order, SRPE was not different between orders. The lack of a clear order by time interaction between T, C, volume-load and SRPE was also observed by Charro and colleagues (2010). Although comparing two different loading schemes, the results from the study by Charro and colleagues showed a nonsignificant change in volume-load, SRPE and T. Charro and colleagues concluded that the lack of a difference in the total exercise volume was the reason for the lack of change between any of the other variables measured such as T. The difference between the current study and design used by Charro and colleagues was that Charro and colleagues purposefully held the volume constant for their two conditions. Conversely, the current study was designed so that volume could increase if T concentrations facilitated potential exercise effort. The current study has

shown that when total volume is the same, the perception of the exercise effort is the same, regardless of how the exercises are ordered during the bout.

#### Confounding Variables That Were Controlled for During the Study

In order to assess the effect of resistance exercise order on circulating T and C concentrations, potential confounding variables were taken into consideration. The potential confounding variables that were controlled included age, body composition, diurnal fluctuations, hydration, sleep, diet and training status. Only the measure of hydration was significantly different between bouts. Changes in hydration status were measured by comparing the body weight of participants for each of the test sessions. There was a significant difference in body weight between session 1 and 2. The significant difference between session 1 and 2 could be as a result of some of the participants not hydrating as part of the 3-hour fast before the resistance training sessions. However, although statistically significant the roughly 1kg difference in mean body weight between session 1 and 2 represented 1.3% increase in bodyweight and therefore the standard of a 2.5% drop of bodyweight used to identify dehydration was not met. Therefore, the lack of a significant order by time interaction for T and C observed in this study can primarily be contributed to exercise order not having an effect on T or C post REB. Although it is possible that as with any study of endocrinology, other unrecognized confounding variables may have influenced the results.

## CHAPTER 5

### SUMMARY, CONCLUSION, AND FUTURE RESEARCH RECOMMENDATIONS

This study was undertaken to examine the effects of resistance exercise order on circulating testosterone (T) and cortisol (C) concentrations along with changes in exercise volume and session rating of perceived exertion. This chapter summarizes the study, forms conclusions from the results, acknowledges the limitations of the study and discusses future recommendations for research.

#### Summary

While the resistance exercise variables intensity, volume, rest period length, mode and repetitions to maximum or failure have all been established as important in optimizing the anabolic milieu for skeletal muscle hypertrophy, exercise order has not been examined. As part of the current view on resistance exercise order, many in the lay literature suggest that performing lower body exercises such as the leg press will produce more of the anabolic hormone T than small muscle mass upper body exercises such as the bench press. Positioning the lower body exercise before the upper body exercise should allow the upper body a greater exposure to T when exercised. However, this assumption had not been tested.

A corollary to the premise that ordering the lower body exercises first in an REB as a means of capitalizing on a more positive anabolic milieu created by the lower body exercises, is that the anabolic milieu might also alter the work accomplished after the lower body exercises. Additionally, the perception of the training load may also change when the upper body skeletal muscles are in the presence of elevated T concentrations resulting from ordering the LB exercises before the UB exercises.

To assess the potential effect of exercise order on circulating T and C, training volume, and the perception of training effort, two resistive exercise sessions utilizing two different resistance exercise orders were performed. The first session used a resistance exercise order of bench press-leg press (UB-LB) and the second session a leg press-bench press (LB-UB) order. Blood samples were taken pre-, mid- and postsession to determine lactate concentration, changes in plasma volume, and T and C responses to the two different orders. A correction for plasma volume provided both a corrected and uncorrected plasma volume value for both T and C. Volume (sets x repetitions) was also recorded for both exercises during both sessions. A session rating of perceived exertion (SRPE) was obtained after both the UB-LB and LB-UB orders.

### Conclusion

Based on a lack of statistical significance, the exercise order of the REBs used in this study does not affect the T and C response to a resistance exercise bout.

### Limitations

While the study design effectively controlled for many of the important variables that could have confounded the results of the study (diet, sleep, hydration etc.) the study

design was limited by two factors. The first was the lack of a randomized or balanced ordering of the upper body-lower body (UB-LB) and lower body-upper body (LB-UB) exercise orders. All 9 participants did the same ordering with UB-LB for session 2 and LB-UB for session 3. However, randomizing the ordering would have placed a greater burden on the participants to perform an additional week of high volume resistance exercise. An additional week would have also exerted a training effect on the participants possibly depressing T values from baseline.

Another potential confounding factor could have been that accumulated fatigue from the UB-LB order could have reduced the exercise volume in LB-UB order. However, the T and C presession values were not significantly different between the UB-LB and LB-UB orders, in support of the assumption that the participants' did not carry any fatigue into the LB-UB order from the UB-LB order. Further more, Nindl and colleagues (2001) concluded that T values are restored after 12-24 hrs, which was well within the 72 hrs allotted between the UB-LB and LB-UB ordered session of this study.

The other potential limitation of the study design was the possible influence of delayed onset muscle soreness (DOMS). Delayed onset of muscle soreness incurred from the UB-LB session may have confounded the training volume recorded during LB-UB session. The majority of the participants did report minor to moderate soreness (2 out of a 5 point scale, 5 being the highest). However, participants did not feel that soreness interfered with their ability to perform repetitions for either the UB-LB and LB-UB orders. The participants' previous experience in resistance training should have protected them from any significant decrements in volume despite minor to moderate soreness (Clarkson et al., 1992). However, it is possible that the total and individual leg press and



or bench press volumes may have been higher in the LB-UB order if a longer wash out period had been observed between the UB-LB and LB-UB sessions. As mentioned, randomizing the ordering of the UB-LB and LB-UB bouts would have eliminated any questions regarding the effect of DOMS on volume in session 3.

Besides the preceding two study design limitations, there were some challenges with blood sample collection. Three of the 9 participants had to redo the bench press from UB-LB session in order to measure hematocrit concentrations. During the original UB-LB session, the capillary tube used to collect blood for a measure of hematocrit did not hold the sample when centrifuged. Lactate concentrations and volume were compared between the original and repeated UB-LB session in order to ensure that hematocrit values were indicative of the values that would have been observed during the original UB-LB session. Despite the replication of the UB-LB session bout there still is the introduction of error into the plasma volume corrected values. One pre time point lactate was also redone because of a malfunction with the lactate analyzer. Additionally, ARUP misplaced one of the premeasurement samples and thus a mean replacement technique was used for this missing time point which may have influenced the results of the statistical analyses.

Other challenges with blood draws did occur on two occasions when the venipuncture was not immediately obtained postresistance exercise because the blood draw technician missed the vein. The delay in one situation was around 2 mins while the other was around 5. Certainly these longer sample collecting periods could influence the timing of the recorded results for T and C concentrations.

A procedural limitation during the study was in the subjective assessment of the recorded repetitions. Each repetition for the bench press and leg press was performed to a metronome cadence. When the participant performed a repetition that was determined to be off cadence the set was completed. While most final repetitions per set were easy to designate as completed or not completed, there were situations where determining if the participant performed a repetition to the metronome cadence was more difficult. Thus it is possible that there was error introduced into the quantification of the amount of repetitions performed per set.

Statistically the study was underpowered for the order by time interaction. However, the partial eta squared values for the interaction effects were weak to modest. To be fully powered with such a small effect would have required over 60 participants. Even if the study was fully powered, a significant result with such a small effect size would mean the results would not be practically significant. Therefore, although the study was underpowered for the order by time interaction the sample size needed to find a significant result would not indicate a practically significant influence of exercise order on the anabolic milieu.

Generalizability of the study is also limited to resistance trained adult males. Additionally, other multiple joint exercises such as the squat or shoulder press may produce different T responses than those seen in the current study. The study was reductionist in approach, only using two resistance exercises which reduced the generalizability to common resistance exercise bouts utilizing multiple exercises and multiple muscle groups throughout the body.

### Future Research Recommendations

This study served as an initial investigation into the effect of exercise order on T and C concentrations and made an assumption based on previous literature that T matters in regard to the development of skeletal muscle hypertrophy, strength and power. However, there have been recent challenges to the importance of T to those variables; therefore, more research must be done to firmly establish the link between T and muscle qualities.

The importance of C in skeletal muscle is also an area where much research is also needed. A recent study (Kramer et al., 2009) has shown that controlling postexercise C concentrations does positively influence muscle hypertrophy. Therefore, C concentrations may be of greater interest in relation to changes in muscle hypertrophy than T, if T is shown to be less of a factor than is currently believed.

If T is determined to be an important hormone in skeletal muscle hypertrophy the combination of exercise order with the other program variables (volume, intensity, and rest period) will also need to be investigated to determine the optimal program configuration to maximize T concentrations. It is possible that performing power exercises such as the power clean may produce greater T concentrations as opposed to the resistance exercises used in this study. It is possible that whole body exercises could also change how lower body and upper body specific exercises affect T and C kinetics.

The study of the effect of resistance exercise order on T concentrations in other populations such as the elderly and elite athletes should also be completed. Athletes with a longer training background may response differently than those that are less trained to a certain exercise order. While this study recruited health young, strength train men, this

sample would not be called a sample of advanced to elite athletes. Conversely, other populations such as the elderly and females may also respond to resistance exercise order differently than do resistance trained males.

Ultimately if the effect of T and C on skeletal muscle size and strength is to be better understood, changes in the androgen and glucocorticoid receptor densities must also be undertaken. Because endocrinology is composed of two components, a ligand and receptor, measuring one without the other can leave an incomplete picture on the importance of the circulating concentration of the hormone and the order by time interaction given the potential variability associated with the up and down regulation of the hormone's receptor. For example, previous research (Spiering et al., 2009) has clearly shown the importance of taking androgen receptor density in to account when deciphering the true affect of T on skeletal muscle. Future studies should seek to measure both T and C and their corresponding receptors when analyzing the potential importance of resistance exercise order on skeletal muscle hypertrophy and strength.

## APPENDIX A

### 3-DAY FOOD RECALL

#### Tips for Keeping an Accurate Food Record

The purpose of the food record is to provide you with an idea of the nutritional quality of your typical diet. In order for it to be meaningful, please follow these guidelines.

1. Record your total intake for at least 3 days, including 2 week days and 1 weekend day. Use days that are fairly typical for you, and try not to alter your intake because someone will be looking at the record. *Total intake* includes all beverages, meals, snacks, bites, sips, and tastes.
2. Be as specific as possible about the foods and drinks that you eat. List condiments, ingredients, preparation methods, and other details.

#### Example

Too vague: Ham sandwich, hot chocolate, peaches

Specific: Ham sandwich—2 slices whole-wheat bread, 4 slices low-salt deli ham, 1 slice cheddar cheese, 2 tsp. mayo, 1 tsp. mustard, 2 leaves of romaine lettuce, 4 slices tomato.  $\frac{3}{4}$  cup hot chocolate made from mix and 1% milk.  $\frac{1}{2}$  cup canned (in light syrup) peaches.

3. Record portion sizes using any approximation you can. Nutrition Facts labels on food packages can help you with these measurements.

#### Measurement Examples

Volume Cups, fluid ounces, tablespoons, 2" x 2" x 1"

Weight Pounds, ounces, grams

Size Small, medium, large

Comparisons About the size of a tennis ball

Count 2 eggs, 15 French fries, 1 slice pizza

Day/Time	Food Item	Amount	Condiment	Amount	Beverage	Amount

$\frac{1}{4}$  cup = golf ball;  $\frac{1}{2}$  cup = tennis or racquet ball; 1 cup = small fist; 1 oz. = one handful or matchbox; 4 oz. fish filet = eyeglass case; 3 oz. portion of cooked meat = a deck of playing cards or cassette tape; 1 teaspoon = quarter or tip of your thumb; 3 teaspoons = 1 tablespoon; 8 fl. oz. = 1 cup

## APPENDIX B

### IRB CONSENT FORM

#### Consent Document

##### **BACKGROUND**

You are being asked to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you want to volunteer to take part in this study. The purpose of the study is to determine whether there is a difference in the testosterone and cortisol response depending on how the order of lower body and upper body resistance training exercises are ordered during a training bout. Additionally, we intent to find out if performing either upper or lower body strength training make the subsequent exercise bout easier or allows the ability to perform more work. The study is being conducted for dissertation research in the department of exercise and sport science.

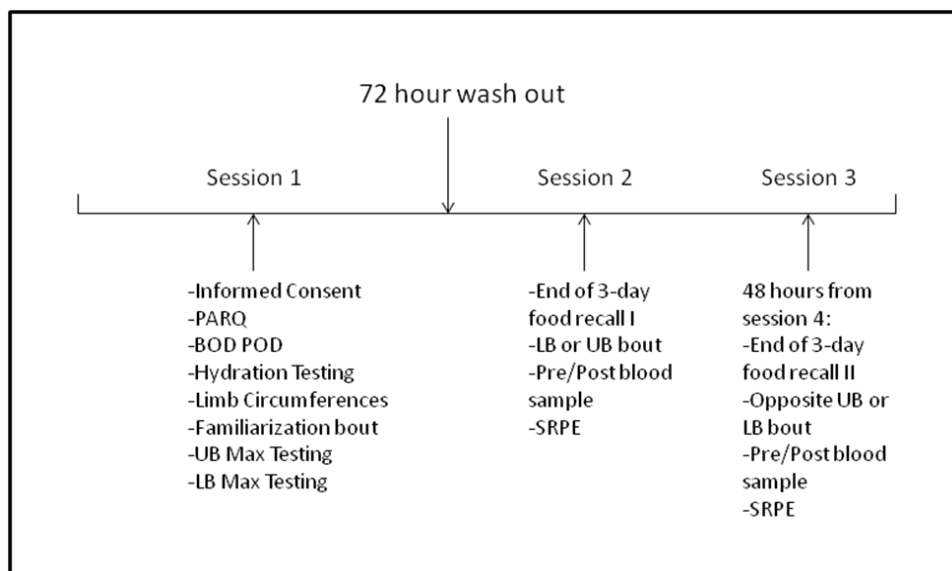
##### **STUDY PROCEDURE**

Before participation you will be required to fill out a health history questionnaire and maintain a consistent diet throughout the duration of the study. Your diet will be checked using two 3-day food recalls over the course of the experimental week. Instructions on how to use the recalls will be given to you. You will be encouraged to keep the same eating and sleep patterns throughout the study. There must not be any supplement or anabolic steroid use at anytime during this study. Supplement use must stop 48 hours before day 1 of the study and steroid use must not have occurred 6 months prior to the session 1. On the first day of the study you will have your height, weight, limb circumferences and body composition assessed followed by a familiarization protocol (Figure 1). Body composition will be assessed using a BOD POD which utilizes air displacement in a sealed chamber for two to three bouts of measurement roughly 45 seconds long to measure body composition and only requires that the participant wears tight fitting clothing such as biking shorts. Hydration status will also be determined one session 1 of the study by a specific gravity strip that will require you to place the strip into the urine stream. You will be provided a closable, lockable bathroom located in the lab for this procedure.

In order to assess testosterone and cortisol, blood sampling will be necessary. Three blood samples will be taken via venipuncture for session 2 and 3 (pre, mid and post exercise), around 15ml per sample for a total around 45ml. For prospective, when donating blood a unit is roughly 450ml, much greater than the amount sampled during this study. A total of 6 venipunctures will be conducted over the 2 days of the study.

Because the study is about hormonal responses to strength training, there is a maximum and experimental strength training protocol that is moderate to vigorous in intensity. Before the maximum and experimental protocols as previously mentioned you will participate in a familiarization protocol on session 1 in order to ensure you have the proper lifting techniques to perform the exercises correctly for the maximum and experimental protocols. The exercises that will be performed are the barbell bench press and leg press. An adequate warm up will be provided before any resistance training. In addition to the familiarization protocol, two maximum strength testing protocols will also be done, one for the bench press and one for the leg press (Figure 1). Maximum strength testing will be done using a predicted protocol which will require maximum effort over 3-10 repetitions with a load that will bring you to the point you can't do another repetition.

Following the familiarization and maximum strength testing in session 1, you will participate in the experimental protocols which will include two resistance training bouts over the course of 72 hours (Figure 1). You will be asked to perform both the upper body-lower body resistance training bout and then 72 hours later the lower body-upper body resistance training bout. All resistance training bouts will require 4 sets of 10 repetitions per exercise with a 1.5 minute rest in between each set and 5 minute rest in between each exercise. Every repetition will be performed to the beat of a metronome. After each bout you will be asked how you felt 30 minutes after each resistance training bout using a scale from 0-10. Total time to complete each strength training bout will be no more than 2 hours.



*Figure 1. Timeline for the Study*

In summary, this study will require 1 week of participation. There are a total of 6 venipunctures, three per day for session 2 and 3 and the experimental exercise protocol is moderate to vigorous in intensity.

## RISKS

The protocol for this study will require moderate to vigorous strength training which could produce muscle soreness or potentially even injury to muscles, ligaments, tendons or bone. Additionally, there may be minor bruising or discomfort associated with the



insertion of the cannula in the lower arm. Effort will be required to perform the exercise protocols which will be strenuous and will be physically challenging.

**BENEFITS**

The aim of this research is to quantify if upper body or lower body strength training is more advantages in promoting increases testosterone. If either body segment produces a greater testosterone response the way in which resistance training is programmed could be influenced.

**CONFIDENTIALITY**

Your data will be kept confidential. Data and records will be stored in a filing cabinet in a locked office or on a password protected computer located in the researcher's work space. Only the researcher and members of his study team will have access to this information. Any publications that may result from this research will not include any personal information.

If you indicate in the health history screening that you are or have used anabolic steroids in the last three months, that information will be confidential, however, you will be excluded from the study.

**PERSON TO CONTACT**

If you have questions, complaints or concerns or feel you have been harmed as a result of participation this study; you can contact Jason Miller at 801-918-4192. Jason is available 24 hours a day; however, if it is not an emergency please call during regular working hours 8am-5pm.

**INSTITUTIONAL REVIEW BOARD**

Contact the Institutional Review Board (IRB) if you have questions regarding your rights as a research participant. Also, contact the IRB if you have questions, complaints or concerns which you do not feel you can discuss with the investigator. The University of Utah IRB may be reached by phone at (801) 581-3655 or by e-mail at [irb@hsc.utah.edu](mailto:irb@hsc.utah.edu). You may also contact the Research Participant Advocate (RPA) by phone at (801) 581-3803 or by email at [participant.advocate@hsc.utah.edu](mailto:participant.advocate@hsc.utah.edu).

**VOLUNTARY PARTICIPATION**

It is up to you to decide whether to take part in this study. Refusal to participate or the decision to withdraw from this research will involve no penalty or loss of benefits to which you are otherwise entitled. However, in order to receive the \$25 compensation the study must be completed. Participating or not participating will not affect your relationship with the investigator.

**COSTS AND COMPENSATION TO PARTICIPANTS**

There is no cost for participation in this study. However, compensation of \$25 will be given upon the completion of the study. Additionally, body composition and maximum strength values will be obtained through the study's protocol which may be useful in assessing personal health and fitness goals.

**CONSENT**

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions. I will be given a signed copy of this consent form. I voluntarily agree to take part in this study.

---

Printed Name of Participant

---

Signature of Participant

---

Date

---

Printed Name of Researcher or Staff

---

Signature of Researcher or Staff

---

Date

## APPENDIX C

### SESSION RATING OF PERCEIVED EXERTION

Rating	Descriptor
0	Rest
1	Very, Very Easy
2	Easy
3	Moderate
4	Somewhat Hard
5	Hard
6	-
7	Very Hard
8	-
9	-
10	Maximal

Modified Session Rating of Perceived Exertion Chart (Dey et al., 2004). Participants are shown the scale 30 minutes after their workout and asked, “How was your workout?” Dey et al., (2004). Monitoring exercise intensity during resistance training using the session RPE Scale. *Journal of Strength and Conditioning Research*, 18(2), 353-358.

## REFERENCES

- Ahtiainen, J. P., Pakarinen, A., Alen, M., Kraemer, W. J., & Häkkinen, K. (2003). Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. *European Journal of Applied Physiology*, 89, 555-563.
- Ahtiainen, J. P., Pakarinen, A., Kraemer, W. J., & Häkkinen, K. (2003). Acute hormonal and neuromuscular responses and recovery to forced vs. maximum repetitions multiple resistance exercises. *International Journal of Sports Medicine*, 24, 410-418.
- Ahtiainen, J. P., Pakarinen, A., Kraemer, W. J., & Häkkinen, K. (2004). Acute hormonal responses to heavy resistance exercise in strength athletes versus nonathletes. *Canadian Journal of Applied Physiology*, 29(5), 527-543.
- Ahtiainen, J. P., Pakarinen, A., Alen, M., Kraemer, W. J., & Häkkinen, K. (2005). Short vs. long rest period between the sets in hypertrophic resistance training: influence on muscle strength, size and hormonal adaptations in trained men. *The Journal of Strength and Conditioning Research*, 19(3), 572-582.
- Ahtiainen, J. P., Hulmi, J. J., Kraemer, W. J., Lehtl, M., Pakarinen, A., Mero, A. A. Karavirta, L., et al. (2009). Strength, endurance or combined training elicit diverse skeletal muscle myosin heavy chain isoform proportion but unaltered androgen receptor concentration in older men. *International Journal of Sports Medicine*, 30, 879-887.
- Adlercreutz, H., Harkonen, M., Kuoppasalmi, K., & Karvonen, J. (1986). Effect of training on plasma anabolic and catabolic steroid hormones and their response during physical exercise. *International Journal of Sports Medicine*, 7(Supp), 27-28.
- Anderson, K. E., Rosner, W., Khan, M. S., New, M. I., Pang, S., Wissel, P. S., & Kappas, A. (1987). Diet-hormone interactions: Protein/carbohydrate ratio alters reciprocally the plasma levels of testosterone and cortisol and their respective binding globulins in man. *Life Sciences*, 40, 1761-1768.
- Baechle, T. R. (1994). *Essentials of strength and conditioning*. Champaign, IL: Human Kinetics.

- Bamman, M. M., Shipp, J. R., Jiang, J., Gower, B. A., Hunter, G. R., Goodman, A., McLafferty, Jr., C. L., et al. (2001). Mechanical load increase muscle IGF-1 and androgen receptor mRNA concentrations in humans. *American Journal of Physiology, Endocrinology and Metabolism*, 280, E383-E390.
- Beaven, C. M., Cook, C. J., & Gill, N. D. (2008). Significant strength gains observed in rugby players after specific resistance exercise protocols based on individual salivary testosterone response. *Journal of Strength and Conditioning Research*, 22(2), 419-425.
- Bhasin, S., Storer, T. W., Berman, N., Callegari, C., Clevenger, B., Phillips, J., Bunnell, T. J., et al. (1996). The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *New England Journal of Medicine*, 335, 1-7.
- Bhasin, S., Woodhouse, L., Casaburi, R., Singh, A. B., Bhasin, D., Berman, N., Chen, X., et al. (2001). Testosterone dose-response relationships in healthy young men. *American Journal of Physiology, Endocrinology and Metabolism*, 281, E1172-E1181.
- Bland, J. M., & Altman, D. G. (1995). Multiple significance tests: The Bonferroni method. *British Medical Journal*, 310, 170.
- Bompa, T. O. (1999). *Periodization training for sport*. Champaign, IL: Human Kinetics.
- Bompa, T. O. (2003). *Serious strength training* (2nd ed.). Champaign, IL: Human Kinetics.
- Borer, K. T. (2003). *Exercise endocrinology*. Champaign, IL: Human Kinetics.
- Bosco, C., Colli, R., Bonomi, R., Von Duvillard, S. P., & Viru, A. (2000). Monitoring strength training: Neuromuscular and hormonal profile. *Medicine and Science in Sports and Exercise*, 32(1), 202-208.
- Braith, R. W., Graves, J. E., Leggett, S. H., & Pollock, M. L. (1993). Effect of training on the relationship between maximal and submaximal strength. *Medicine and Science in sports and exercise*, 25(1), 132-138.
- Brandenberger, G., & Follenius, M. (1975). Influence of timing and intensity of muscular exercise on temporal patterns of plasma cortisol levels. *Journal of Clinical Endocrinology and Metabolism*, 40, 845.
- Brandenberger, G., Follenius, M., & Hietter, B. (1982). Feedback from meal-related peaks determines diurnal changes in cortisol response to exercise. *Journal of Clinical Endocrinology and Metabolism*, 54(3), 592-596.

- Bremner, W. J., Vitiello, M. V., & Prinz, P. N. (1983). Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. *Journal of Clinical Endocrinology and Metabolism*, 56(6), 1278-1281.
- Breuner, C., & Orchinik, M. (2002). Plasma binding proteins as mediators of corticosteroid action in vertebrates. *Journal of Endocrinology*, 175, 99-112.
- Brzycki, M. (1993). Strength testing-Predicting a one-rep max from reps-to-fatigue. *Journal of Physical Education, Recreation and Dance*, 64(1), 88-90.
- Buresh, R., Berg, K., & French, J. (2009). The effect of resistive exercise rest interval on hormonal response, strength, and hypertrophy with training. *The Journal of Strength and Conditioning Research*, 23(1), 62-71.
- Chance, J., Berube, J., Vandersmissen, M., & Blanckaert, N. (2009). Evaluation of BD vacutainer PST II blood collection tube for special chemistry analytes. *Clinical Chemistry Laboratory Methods*, 47(3), 358-361.
- Charro, M. A., Aoki, M. S., Coutts, A. J., Araujo, R. C., & Bacurau, R. F. (2010). Hormonal, metabolic and perceptual responses to different resistance training systems. *Journal of Sports Medicine and Physical Fitness*, 50, 229-234.
- Clarkson, P. M., Nosaka, K., & Braun, B. (1992). Muscle function after exercise-induced muscle damage and rapid adaptation. *Medicine and Science in Sports and Medicine*, 24(5), 512-520.
- Crewther, B., Cronin, J., Keogh, J., & Cook C. (2008). The salivary testosterone and cortisol response to three loading schemes. *The Journal of Strength and Conditioning Research*, 22(1), 250-255.
- Cumming, D. C., Wall, S. R., Galbraith, M. A., & Belcastro, A. N. (1987). Reproductive hormone responses to resistance exercise. *Medicine and Science in Sports and Exercise*, 19(3), 234-238.
- David, M. (2007). How to increase your flat bench press by doing squats. *Criticalbench.com*. <http://www.criticalbench.com/flat-bench-press.htm>
- Dey, M. L., McGuigan, M. R., Brice, G., & Foster, C. (2004). Monitoring exercise intensity during resistance training using the session RPE scale. *Journal of Strength and Conditioning Research*, 18(2), 353-358.
- Dhillon, W. S., Kong, W. M., Le Roux, C. W., Alaghband-Zadeh, J., Jones, J., Carter, G., Mendoza, N., et al. (2002). Cortisol-binding globulin is important in the interpretation of dynamic test of the hypothalamic-pituitary-adrenal axis. *European Journal of Endocrinology*, 146, 231-235.

- Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, 37(2), 247-248.
- Doerr, P., & Pirke, K. M. (1976). Cortisol-induced suppression of plasma testosterone in normal adult males. *Journal of Clinical Endocrinology and Metabolism*, 43, 622.
- Durand, R. J., Castracane, V. D., Hallander, D. B., Tryniecki, J. L., Bamman, M. M., O'Neal, S., Hebert, E. P., et al., (2003). Hormonal responses from concentric and eccentric muscle contractions. *Medicine & Science in Sports & Exercise*, 35(6), 937-943.
- Fahey, T. D., Rolph, R., Moungmee, P., Nagel, J., & Mortara, S. (1976). Serum testosterone, body composition, and strength of young adults. *Medicine & Science in Sports & Exercise*, 8(1), 31-34.
- Fitzgerald, M. (2003). Three must do upper body muscle builders: to develop the ultimate top half, you have to work your lower body. *Men's Fitness, Feb.* Retrieved from [http://findarticles.com/p/articles/mi\\_m1608/is\\_2\\_19/ai\\_96621185/](http://findarticles.com/p/articles/mi_m1608/is_2_19/ai_96621185/)
- Foster, C., Daines, E., Hector, L., Snyder, A. C., & Welsh, R. (1996). Athletic performance in relation to training load. *Wisconsin Medical Journal*, June, 370-374.
- Foster, C., Florhaug, J. A., Franklin, J., Gottschall, L., Hrovatin, L. A., Parker, S., Doleshal, P., et al. (2001). A new approach to monitoring exercise training. *Journal of Strength and Conditioning Research*, 15(1), 109-115.
- French, D. N., Kraemer, W. J., Volek, J. S., Spiering, B. A., Judelson, D. A., Hoffman, J. R., & Maresh, C. M. (2007). Anticipatory responses of catecholamines on muscle force production. *Journal of Applied Physiology*, 102, 94-102.
- Fry, A. C., Kraemer, W. J., Stone, M. H., Koziris, L. P., Thrush, J. T., & Fleck, S. J. (2000). Relationships between serum testosterone, cortisol, and weightlifting performance. *Journal of Strength and Conditioning Research*, 14, 338-343.
- Gayard, V., Alvinerie, M., & Toutain, P. L. (1996). Interspecies variations of corticosteroid-binding globulin parameters. *Domestic Animal Endocrinology*, 13(1), 35-45.
- Goto, K., Ishii, N., Kizuka, T., & Takamatsu, K. (2005). The impact of metabolic stress on hormonal responses and muscular adaptations. *Medicine and Science and Sports and Exercise*, 37(6), 955-963.
- Gotshalk, L. A., Loebel, C. C., Nindl, B. C., Putukian, M., Sebastianelli, W. J., Newton, R. U., Häkkinen, K., et al., (1997). Hormonal responses of multiset versus single-set

- heavy-resistance exercise protocols. *Canadian Journal of Applied Physiology*, 22(3), 244-255.
- Guezennec, Y., Leger, L., Lhoste, F., Aymonod, M., & Pesquies, P. C. (1986). Hormone and metabolite response to weight-lifting training sessions. *International Journal of Sports Medicine*, 7, 100-105.
- Häkkinen, K., Pakarinen, A., Alén, M., & Komi, P. V. (1985). Serum hormones during prolonged training of neuromuscular performance. *European Journal of Applied Physiology*, 53, 287-293.
- Häkkinen, K., Pakarinen, A., Alén, M., Kauhanen, H., & Komi, P. V. (1987). Relationships between training volume, physical performance capacity, and serum hormone concentrations during prolonged training in elite weight lifters. *International Journal of Sports Medicine*, 8(Supp), 61-65.
- Häkkinen, K., Pakarinen, A., Alén, M., Kauhanen, H., & Komi, P. V. (1988). Neuromuscular and hormonal adaptations in athletes to strength training in two years. *Journal of Applied Physiology*, 65(6), 2406-2412.
- Häkkinen, K., & Pakarinen, A. (1993). Acute hormonal responses to two different fatiguing heavy-resistance protocol in male athletes. *The Journal of Applied Physiology*, 74(2), 882-887.
- Häkkinen, K., & Pakarinen, A. (1995). Acute hormonal responses to heavy resistance exercise in men and women at different ages. *International Journal of Sports Medicine*, 16, 507-513.
- Häkkinen, K., Pakarinen, Newton, R. U., & Kraemer, W. J. (1998). Acute hormone responses to heavy resistance lower and upper extremity exercise in young versus old men. *European Journal of Applied Physiology*, 77, 312-319.
- Hammes, A., Andreassen, T. K., Spoelgen, R., Raila, J., Hubner, N., Schulz, H., Metzger, J., et al. (2005). Role of endocytosis in cellular uptake of sex steroids. *Cell*, 122, 751-762.
- Hansen, S., Kvorning, T., Kjær, M., & Sjøgaard, G. (2001). The effect of short-term strength training on human skeletal muscle: the importance of physiologically elevated hormone levels. *Scandinavian Journal of Medicine & Science in Sports*, 11, 347-354.
- Hatfield, F. C. H. (1989). *Power*. Chicago, IL: Contemporary Books.
- Hayes, F. J. (2000). Editorial: Testosterone-foundation of youth or drug of abuse? *The Journal of Clinical Endocrinology & Metabolism*, 85(9), 3020-3023.



- Heyns, W., & Coolens, J. L. (1988). Physiology of corticosteroid-binding globulin in humans. *Annals New York Academy of Sciences*, 538, 122-9.
- Heyward, V. H. (2006). *Advanced fitness assessment and exercise prescription* (5th ed). Champaign, IL: Human Kinetics.
- Hickson, R. C., Czerwinski, S. M., Falduto, M. T., & Young, A. P. (1990). Glucocorticoid antagonism by exercise and androgenic-anabolic steroids. *Medicine and Science in Sports and Exercise*, 22(3), 331-340.
- Hoffman, J. R., Im, J., Rundell, K. W., Kang, J., Nioka, S., Speiring, B. A., Kime, R., et al. (2003). Effect of muscle oxygenation during resistance exercise on anabolic hormone response. *Medicine and Science in Sports and Exercise*, 35(11), 1929-1934.
- Hoffman, J. R., Kraemer, W. J., Bhasin, S., Storer, T., Ratamess, N. A., Haff, G. G., Willoughby, D. S., et al., (2009). Position stand on androgen and human growth hormone use. *Journal of Strength and Conditioning Research*, 23(Supp.), S1-S59.
- Horton, R. (1978). Sex steroid production and secretion in the male. *Andrologia*, 10(3), 183-194.
- Hsueh, A. J. W., Dahl, K. D., Vaughan, J., Tucker, E., Rivier, J., Bardin, C. W., & Vale, W. (1987). Heterodimers and homodimers of inhibin subunits have different paracrine action in the modulation of luteinizing hormone-stimulated androgen biosynthesis. *Proceedings of the National Academy of Sciences*, 84, 5082-5086.
- Hryb, D. J., Khan, M. S., Romas, N. A., & Rosner, W. (1986). Specific binding of human corticosteroid-binding globulin to cell membranes. *Proceedings of the National Academy of Science USA*, 83, 3253-3256.
- Heyns, W., & Coolens, J. L. (1988). Physiology of corticosteroid-binding globulin in humans. *Annals of the New York Academy of Sciences*, 138, 122-129.
- Izquierdo, M., Häkkinen, K., Ibañez, J., Garrues, M., Antón, A., Zúñiga, Larrión, J. L., et al. (2001). Effects of strength training on muscle power and serum hormones in middle-aged and older men. *Journal of Applied Physiology*, 90, 1497-1507.
- Ježová, D., & Vigaš, M. (1981). Testosterone response to exercise during blockade and stimulation of adrenergic receptors in man. *Hormone Research*, 15, 141-147.
- Ježová, D., Vigaš, M., Tatár, P., Kvetňanský, Nazar, K., Kaciuba-Uścilko, H., & Kozolowski, S. (1985). Plasma testosterone and catecholamine responses to physical exercise of different intensities in men. *European Journal of Applied Physiology*, 54, 64-66.

- Johnson, P. (2010). Squats and deadlifts release testosterone and growth hormone. *Rate My Arms*. Retrieved from <http://www.ratemyarms.com/training/squats-deadlifts-release-testosterone-and-growth-hormone/>
- Judelson, D. A., Maresh, C. M., Yamamoto, L. M., Farrell, M. J., Armstrong, L. E., Kraemer, W. J., Volek, J. S., et al. (2008). Effect of hydration state on resistance exercise-induced endocrine markers of anabolism, catabolism, and metabolism. *Journal of Applied Physiology*, 105, 816-824.
- Jürimäe, T., Karelson, K., Smirnova, T., & Viru, A. (1990). The effect of a single-circuit weight-training session on the blood biochemistry of untrained university students. *European Journal of Applied Physiology*, 61, 344-348.
- Kadi, F., Bonnerud, P., Eriksson, A., & Thornell, L. E. (2000). The expression of androgen receptors in human neck and limb muscles: effects of training and self-administration of androgenic-anabolic steroids. *Histochemistry Cellular Biology*, 113, 25-29.
- Kraemer, R. R., Kilgore, J. L., Kraemer, G. R., & Castracane, D. V. (1992). Growth hormone, IGF-1, and testosterone responses to resistive exercise. *Medicine and Science in Sports and Exercise*, 24, 1346-1352.
- Kraemer, R. R., Kilgore, J. L., & Kraemer, G. R. (1993). Plasma volume changes in response to resistive exercise. *Journal of Sports Medicine and Physical Fitness*, 33, 246-251.
- Kraemer, R. R., Hollander, D. B., Reeves, G. V., Francois, M., Ramadan, Z. G., Meeker, B., Tryniecki, J. L., et al. (2006). Similar hormonal responses to concentric and eccentric muscle actions using relative loading. *European Journal of Applied Physiology*, 96, 551-557.
- Kraemer, W. J., Noble, B. J., Clark, M. J., & Culver, B. W. (1987). Physiologic responses to heavy-resistance exercise with very short rest periods. *International Journal of Sports Medicine*, 8, 247-252.
- Kraemer, W. J., Marchitelli, L., Gordon, S. E., Harman, E., Dziados, J. E., Mello, R., Frykman, P., et al. (1990). Hormonal and growth factor responses to heavy resistance training exercise protocols. *Journal of Applied Physiology*, 69(4), 1442-1450.
- Kraemer, W. J., Gordon, S. E., Fleck, S. J., Marchitelli, L. J., Mello, R., Dziados, J. E., Friedl, K., et al. (1991). Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *International Journal of Sports Medicine*, 12, 228-235.

- Kraemer, W. J., Fry, A. C., Warren, B. J., Stone, M. H., Fleck, S. J., Kearney, Conroy, B. P., et al. (1992). Acute hormonal responses in elite junior weightlifters. *International Journal of Sports Medicine*, 13, 103-109.
- Kraemer, W. J., Staron, R. S., Hagerman, F. C., Hikida, R. S., Fry, A. C., Gordon, S. E., Nindl, B. C., et al. (1998). The effects of short-term resistance training on endocrine function in men and women. *European Journal of Applied Physiology*, 78, 69-76.
- Kraemer, W. J., Volek, J. S., Bush, J. A., Putukian, M., & Sebastianelli, W. J. (1998). Hormonal responses to consecutive days of heavy-resistance exercise with or without nutritional supplementation. *Journal of Applied Physiology*, 85, 1544-1555.
- Kraemer, W. J., Koziris, L. P., Ratamess, N. A., Häkkinen, K., Triplett-McBride, N. T., Fry, A. C., Gordon, S. E., et al. (2002). Detraining produces minimal changes in physical performance and hormonal variables in recreationally strength-trained men. *Journal of Strength and Conditioning Research*, 16(3), 373-382.
- Kraemer, W. J., French, D. N., Paxton, N. J., Häkkinen, K., Volek, J. S., Sebastianelli, W. J., Putukian, M., et al. (2004). Changes in exercise performance and hormonal concentrations over a big ten soccer season in starters and nonstarters. *Journal of Strength and Conditioning Research*, 18(1), 121-128.
- Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Medicine*, 35(4), 339-361.
- Kraemer, W. J., & Rogol, A. D. (2005). *The endocrine system in sports and exercise*. Malden, MA: Blackwell Publishing.
- Kraemer, W. J., Ratamas, N. A., Volek, J. S., Häkkinen, K., Rubin, M. R., French, D. N., Gómez, A. L., et al. (2006). The effects of amino acid supplementation on hormonal responses to resistance training overreaching. *Metabolism Clinical and Experimental*, 55, 282-291.
- Kraemer, W. J., Spiering, B. A., Volek, J. S., Ratamess, N. A., Sharman, M. J., Rubin, M. R., French, D. N., et al. (2006). Androgenic responses to resistance exercise: Effects of feeding and L-carnitine. *Medicine and Science in Sports and Exercise*, 38(7), 1288-1296.
- Kraemer, W. J., Hatfield, D. L., Volek, J. S., Fragala, M. S., Vingren, J. L., Anderson, J. M., Spiering, B. A., et al. (2009). Effects of amino acids supplement on physiological adaptations to resistance training. *Medicine & Science in Sports & Exercise*, 41(5), 1111-1121.

- Kvorning, T., Andersen, M., Brixen, K., & Madsen, K. (2006). Suppression of endogenous testosterone production attenuates the response to strength training: A randomized, placebo-controlled, and blinded intervention study. *American Journal of Physiology, Endocrinology and Metabolism*, 291, E1325-E1332.
- Kvorning, T., Bagger, M., Caserotti, P., & Madsen, K. (2006). Effects of vibration and resistance training on neuromuscular and hormonal measures. *European Journal of Applied Physiology*, 96, 615-625.
- de Leva, P. (1996). Adjustments to Zatisorsky-Seluyanov's segment inertia parameters. *Journal of Biomechanics*, 29(9), 1223-1230.
- Lin, H., Wang, S. W., Wang, R. Y., & Wang, P. S. (2001). Stimulatory effect of lactate on testosterone production by rat Leydig cells. *Journal of Cellular Biochemistry*, 83(1), 147-154.
- Lu, S. S., Lau, C. P., Tung, Y. F., Huang, S. W., Chen, Y. H., Shih, H. C., Tsai, S. C., et al. (1997). Lactate and the effects of exercise on testosterone secretion: evidence for the involvement of a cAMP-mediate mechanism. *Medicine & Science in Sports & Exercise*, 29(8), 1048-1054.
- Madarame, H., Sasaki, K., & Ishii, N. (2010). Endocrine responses to upper- and lower-limb resistance exercises with blood flow restriction. *Acta Physiologica Hungarica*, 97(2), 192-200.
- Mayer, M., & Rosen, F. (1975). Interaction of anabolic steroids with glucocorticoid receptor sites in rat muscle cytosol. *American Journal of Physiology*, 229(5), 1381-1386.
- McCall, G. E., Brynes, W. C., Fleck, S. J., Dickinson, A., & Kraemer, W. J. (1999). Acute and chronic hormonal responses to resistance training designed to promote muscle hypertrophy. *Canadian Journal of Applied Physiology*, 24(1), 96-107.
- Nelson, A. G., & Kokkonen, J. (2001). Acute ballistic muscle stretching inhibits maximal strength performance. *Research Quarterly for Exercise and Sport*, 72(4), 415-419.
- Nindl, B. C., Kraemer, W. J., Deaver, D. R., Peters, J. L., Marx, J. O., Heckman, J. T. & Loomis, G. A. (2001). LH secretion and testosterone concentrations are blunted after resistance exercise in men. *Journal of Applied Physiology*, 91, 1251-1258.
- Pitkänen, H., Mero, A., Oja, S. S., Komi, P. V., Rusko, H., Nummela, A., Saransaari, P., et al. (2002). Effects of training on the exercise-induced changes in serum amino acids and hormones. *Journal of Strength and Conditioning Research*, 16(3), 390-398.

- Purnell, J. Q., Brandon, D. D., Lorne, M. I., Loriaux, D. L., & Samuels, M. H. (2004). Association of 24-hour cortisol production rates, cortisol-binding globulin, and plasma-free cortisol levels with body composition, leptin levels, and aging in adult men and women. *The Journal of Clinical Endocrinology & Metabolism*, 89(1), 281-287.
- Raastad, T., Glomshelter, T., Bjørø, T., & Hallén, J. (2001). Changes in human skeletal muscle contractility and hormone status during 2 weeks of heavy strength training. *European Journal of Applied Physiology*, 84, 54-63.
- Rosner, W., Auchus, R. J., Azziz, R., Sluss, P. M., & Raff, H. (2007). Position statement: Utility, limitations, and pitfalls in measuring testosterone: An endocrine society position statement. *The Journal of Clinical Endocrinology & Metabolism*, 92(2), 405-413.
- Santtila, M., Kyröläinen, H., & Häkkinen, K. (2009). Serum hormones in soldiers after basic training: Effect of added strength or endurance regimens. *Aviation Space and Environmental Medicine*, 80, 615-620.
- Schwab, R. S., Johnson, G. O., Housh, T. J., Kinder, J. E., & Weir, J. P. (1993). Acute effects of different intensities of weight lifting on serum testosterone. *Medicine & Science in Sports & Exercise*, 25(12), 1381-1385.
- Sinha-Hikim, I., Artaza, J., Woodhouse, L., Gonzalez-Cadavid, N., Singh, A.B., Lee, M.I., Storer, T. W., et al. (2002). Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. *American Journal of Physiology, Endocrinology and Metabolism*, 283, 154-164.
- Smilios, I., Pilianidis, T., Karamouzis, M., & Tokmakidis, S. P. (2003). Hormonal responses after various resistance exercise protocols. *Medicine and Science in Sports and Exercise*, 35(4), 644-654.
- Smilios, I., Pilianidis, T., Karamouzis, M., Parlavantzas, A., & Tokmakidis, S. P. (2007). Hormonal responses after a strength endurance resistance exercise protocol in young and elderly males. *International Journal of Sports Medicine*, 28, 401-406.
- Spiering, B. A., Kraemer, W. J., Vingren, J. L., Ratamess, N. A., Anderson, J. M., Armstrong, L. E., Nindl, B. C., et al. (2009). Elevated endogenous testosterone concentrations potentiate muscle androgen receptor responses to resistance exercise. *Journal of Steroid Biochemistry and Molecular Biology*, 114, 195-199.
- Spratt, D. I., O'Dea, L. S., Schoenfeld, D., Butler, J., Narashimha Rao, P., & Crowley, W. F. (1988). Neuroendocrine-gonadal axis in men: Frequent sampling of LH, FSH, and testosterone. *Endocrinology and Metabolism*, 17, E658-E666.

- Spreuwenberg, L. P. B., Kraemer, W. J., Spiering, B. A., Volek, J. S., Hatfield, D. L., Silvestre, R., Vingren, J. L., et al. (2006). Influence of exercise order in a resistance-training exercise session. *Journal of Strength and Conditioning Research*, 20(1), 141-144.
- Staron, R. S., Karapondo, D. L., Kraemer, W. J., Fry, A. C., Gordon, S. E., Falkel, J. E., Hagerman, F. C., et al. (1994). Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *Journal of Applied Physiology*, 76(3), 1247-1255.
- Strel'chyonok, O. A., & Avvakumov, G. V. (1991). Interaction of human CBG with cell membranes. *Journal of Steroid Biochemistry*, 40, 4-6.
- Tarpenning, K. M., Wiswell, R. A., Hawkins, S. A., & Marcell, T. J. (2001). Influence of weight training exercise and modification of hormonal response on skeletal muscle growth. *Journal of Science and Medicine in Sport*, 4(4), 431-446.
- Thuma, J. R., Gilders, R., Verdun, M., & Loucks, A. B. (1995). Circadian rhythm of cortisol confounds responses to exercise: implications for future research. *Journal of Applied Physiology*, 78(5), 1657-1664.
- Tremblay, M. S., Copeland, J. L., & Van Helder, W. (2004). Effect of training status and exercise mode on endogenous steroid hormones in men. *Journal of Applied Physiology*, 96, 531-539.
- Veldhuis, J. D., King, J. C., Urban, R. J., Rogol, A. D., Evans, W. S., Kolp, L. A. & Johnson, M. L. (1987). Operating characteristics of the male hypothalamo-pituitary-gonadal axis: pulsatile release of testosterone and follicle-stimulating hormone and their temporal coupling with luteinizing hormone. *Journal of Clinical Endocrinology and Metabolism*, 65, 929-941.
- Vettor, R., De Pergola, G., Pagano, C., Englaro, P., Laudadio, E., Giorgino, F., Blum, W. F., et al. (1997). Gender difference in serum leptin in obese people: Relationship with testosterone, body fat distribution and insulin sensitivity. *European Journal of Clinical Investigation*, 27, 1016-1024.
- Vingren, J. L., Kraemer, W. J., Hatfield, D. L., Anderson, J. M., Volek, J. S., Ratamass, N. A., Thomas, G. A., et al. (2008). Effect of resistance exercise on muscle steroidogenesis. *Journal of Applied Physiology*, 105, 1754-1760.
- Vingren, J. L., Kraemer, W. J., Hatfield, D. L., Volek, J. S., Ratamass, N. A., Anderson, J. M., Häkkinen, K., et al. (2009). Effect of resistance exercise on muscle steroid receptor protein content in strength-trained men and women. *Steroids*, 74, 1033-1039.

- Viru, A., & Smirnova, K. T. (1992). Stability and variability in hormonal responses to prolonged exercise. *International Journal of Sports Medicine*, 13(3), 230-235.
- Volek, J. S., Kraemer, W. J., Bush, J. A., Incledon, T., & Boetes, M. (1997). Testosterone and cortisol relationship to dietary nutrients and resistance exercise. *Journal of Applied Physiology*, 82(1), 49-54.
- West, D. W. D., Burd, N. A., Tang, J. E., Moore, D. R., Staples, A. W., Holwerda, A. M., Baker, S. K., et al. (2010). Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *Journal of Applied Physiology*, 108, 60-67.
- West, D. W. D., Burd, N. A., Staples, A. W., & Phillips, S. M. (2010). Human exercise-mediated skeletal muscle hypertrophy is an intrinsic process. *The International Journal of Biochemistry & Cell Biology*, ahead of print.
- Wilkinson, S. B., Tarnopolsky, M. A., Grant, E. J., Correia, C. E., & Phillips, S. M. (2006). Hypertrophy with unilateral resistance exercise occurs without increases in endogenous anabolic hormone concentration. *European Journal of Applied Physiology*, 98, 546-555.
- Willoughby, D. S., & Taylor, L. (2004). Effects of sequential bouts of resistance exercise on androgen receptor expression. *Medicine and Science in Sports and Exercise*, 36(9), 1499-1506.
- Yarrow, J. F., Borsa, P. A., Borst, S. E., Sitren, H. S., Stevens, B. R., & White, L. J. (2007). Neuroendocrine responses to an acute bout of eccentric-enhanced resistance exercise bout. *Medicine and Science in Sports and Exercise*, 39(6), 941-947.
- Yarrow, J. F., Borsa, P. A., Borst, S. E., Sitren, H. S., Stevens, B. R., & White, L. J. (2008). Early-phase neuroendocrine responses and strength adaptations following eccentric-enhanced resistance training. *Journal of Strength and Conditioning Research*, 22, 1205-1214.
- Zatvars, V. M., & Kraemer, W. J. (2006). *Science and practice of strength training* (2nd ed). Champaign, IL: Human Kinetics.
- Zhou, Z. X., Lane, M. V., Kemppainen, J. A., French, F. S., & Wilson, E. M. (1995). Specificity of ligand-dependent androgen receptor stabilization: Receptor domain interactions influence ligand dissociation and receptor stability. *Molecular Endocrinology* 9, 208-218.